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THE EFFECT OF HEAT AND CHEMICAL STERILIZATION ON THE RUBBER PARTS OF MILKING MACHINES

J. L. HENDERSON,[1] C. L. ROADHOUSE,[2] AND A. FOLGER[3]

University of California, College of Agriculture, Davis, California

The literature does not cover completely the effect of heat and of the common chemical sterilizing agents on the life (period of usefulness) of the rubber parts of milking machines.[4] In some milk control areas, the law requires heat sterilization of all dairy equipment, including the rubber parts of milking machines. Because such requirements exist and because there is little information concerning the effect of both heat and chemical sterilization on the life of the rubbers, a detailed study of this subject was included in an investigation of the sanitary operation of milking machines.[5] The data reported are based on the operation of four units of a milking machine of one manufacturer[6] which were used for a period of two years in milking the Experiment Station herd.

DETERMINATION OF THE LIFE OF THE RUBBERS

The life of milking machine rubbers depends on several factors, such as quality and age, thoroughness of cleaning, and method of sterilization. In this investigation, factors influencing the life of the rubbers were kept as constant as possible.

Rubber tubes and teat cup liners were assumed to be worn out when they no longer gave efficient service. Air tubes and short milk tubes were discarded when cracked or so stretched at the ends that they would no longer stay on the claws or metal teat cup shells. Teat cup liners were dis-

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[1] Associate in the Experiment Station.

[2] Professor of Dairy Industry and Dairy Technologist in the Experiment Station.

[3] Supervisor of Official Advanced Registry Tests in the Division of Animal Husbandry.

[4] Burgwald, L. H. Cleaning milking machines. U. S. Department of Agriculture Farmers' Bul. 1315. 1923.

[5] Henderson, J. L., Roadhouse, C. L., and Folger, A. The sanitary care, operation and installation of milking machines. California Agr. Ext. Serv. Circ. 69. November, 1932.

[6] The De Laval machine was used in these experiments. It is recognized that there is a variation in the effect of heat upon the life of rubber parts for milking machines.

carded when they no longer were elastic enough to draw the milk rapidly or to stay on the cow's teats. Occasionally they became cracked and were discarded. Throughout the experiment the discarding of rubbers was decided upon by the same two persons in order to maintain a uniform standard. Some dairymen might use the teat cup liners longer than the standard established in this investigation, but the rapidity of milking would be retarded thereby.

The life of the long milk tubes depends largely on the number of times they are stepped on by the cows or are trimmed at the ends. With proper care, they lasted approximately one year with both heat and chemical sterilization.

EFFECT OF HEAT STERILIZATION ON LIFE OF RUBBERS

Procedure.—After each operation, the milking machines were rinsed, taken apart, and brushed according to standard procedures.[5] Once each week the teat cup liners were removed from the metal shells for thorough cleaning and inspection.

The heating was carried out in a rectangular galvanized iron tank, inside which was placed a sloping metal tray, held above the bottom of the tank by metal supports (figure 1). Beneath the tray was a perforated

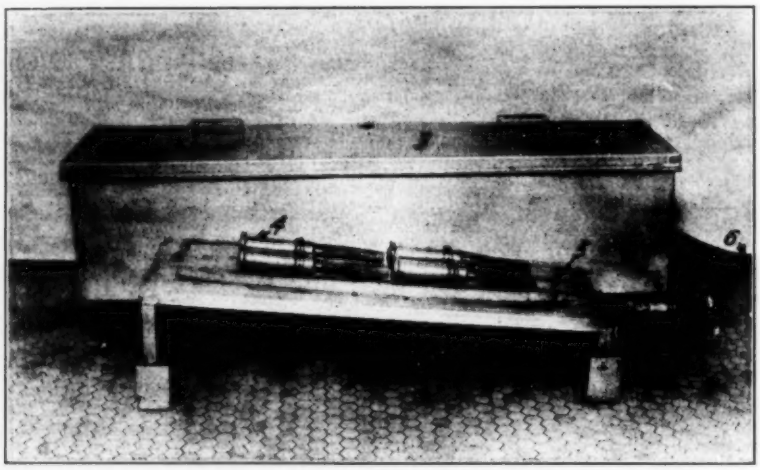

FIG. 1. TANK USED FOR HEAT STERILIZATION.

1. Galvanized iron tank 12 × 12 × 50 inches. 2. Corrugated iron tray used to hold rubber parts. The sloping surface prevents air pockets in the long tubes and permits them to drain when the water is drained from the tank. 3. Cover of tank with hole at center for inserting a recording thermometer. 4. Assembled teat cups on tray. 5. Long milk tube on tray. 6. Steam connection to tank. 7. Drain pipe.

steam pipe, used for heating the water. In this tank the rubber parts were covered with sufficient water to maintain the desired temperature throughout the holding period. A metal cover was kept in place to assist in maintaining the temperature and to protect the rubbers from contamination after the water was drawn from the tank following sterilization.

The temperature of heating and the length of time the heat was applied were checked throughout the experiment by a recording thermometer. The heat was applied in water according to the following procedures:

1. Heating to 170° F., holding at that temperature for 20 minutes, and allowing the rubbers to remain in the heated water and to cool gradually until the next milking time.
2. Heating to 170° F., holding at that temperature for 20 minutes, and immediately drawing the water from the tank.
3. Heating to 185° F., and then, without applying further heat, allowing the rubbers to remain in the water for 20 minutes before draining the tank.

TABLE 1

The effect of heat and chlorine sterilization on the rubber parts of milking machines
(Machines were used and sterilized twice daily)

	170° F. FOR 20 MIN. LEFT IN WATER BETWEEN MILKINGS	170° F. FOR 20 MIN. WATER THEN DRAINED FROM TANK	185° F.—NO MORE HEAT ADDED. WATER DRAINED FROM TANK AFTER 20 MIN.	CHLORINE 200 P.P.M. IN STERILIZING RACK. FRESH SOLUTION PREPARED DAILY
Teat Cup Liners				
Life in weeks	8	9	8	12
Number of sterilizations	112	126	112	168
Number of cow-hours operated	729	819	729	1,092
Number of sets of rubbers used in determining average life	6	4	6	4
Short Milk Tubes				
Life in weeks	10	19	16	18
Number of sterilizations	140	266	224	252
Number of cow-hours operated	910	1,729	1,456	1,638
Number of sets of rubbers used in determining average life	4	4	2	2
Short Air Tubes				
Life in weeks	20	24	23	25 per cent replacements after 30 weeks' use
Number of sterilizations	280	336	322	
Number of cow-hours operated	1,820	2,184	2,093	
Number of sets of rubbers used in determining average life	2	2	2	

The effect of these methods of sterilization on the life of the rubber parts is shown in table 1. The machines were operated on an average of 91 cow-hours per unit per week.

Bacterial determinations of the sterile water rinsings of the assembled rubber parts and claws were prepared to check the efficiency of the various sterilization methods. The bacterial counts were satisfactory except when a temperature of 170° F. was used. At this temperature, thermophilic bacteria developed, especially when the rubbers were left in the water until the next milking time.

Results.—The heating of the milking machine rubber parts to 170° F., maintaining that temperature for 20 minutes, and then draining the water from the tank, showed an increased life of the rubber parts as compared with the method of heating and allowing the rubbers to remain in the water until the next milking time. The third method of heating the rubbers (that is, to 185° F.), holding for 20 minutes without further application of heat, and then draining the tank, reduced the life of the rubber parts very slightly when compared with 170° F. for 20 minutes. This method offers the advantages of requiring only one application of heat and of reducing slightly more the bacterial count in the sterile rinse water used to check the efficiency of sterilization. Furthermore, the thermophilic type of organisms was eliminated by this method, as shown in table 3.

Chlorine sterilization of the milking machines used twice a day was also investigated. Table 1 shows that with chlorine sterilization the life of the teat cup liners was 12 weeks; with heat sterilization, only 8 weeks. The strength of the chlorine solution used and its method of application are described under the procedure for chemical sterilization.

EFFECT OF CHEMICAL STERILIZATION ON LIFE OF RUBBERS

Procedure.—In the application of chemical sterilizers, the cleansing of the rubber parts was the same as that described under heat sterilization. The claws, teat cups, and rubber parts were then assembled ready for use and were placed on the sterilizing rack commonly recommended for chemical sterilization (figure 2). The milking machines were operated and sterilized three times daily throughout the tests and were operated on an average of 252 cow-hours per unit per week.

The hypochlorite solution was prepared as follows: one twelve-ounce can of chloride of lime was mixed with one gallon of water, covered, and allowed to stand in an earthen crock for fifteen hours. Then one hundred grams of tri-sodium phosphate was added to the filtered solution to render the solution more stable and to decrease corrosiveness. This stock solution was tested for strength by Fay's method,[7] and working solutions were prepared each day with water to give a strength of 200 p.p.m.

[7] Fay, A. C. Preparation, testing, and use of chlorine disinfectants. Kansas Agr. Exp. Sta. Circ. 160: 1–8. 1931.

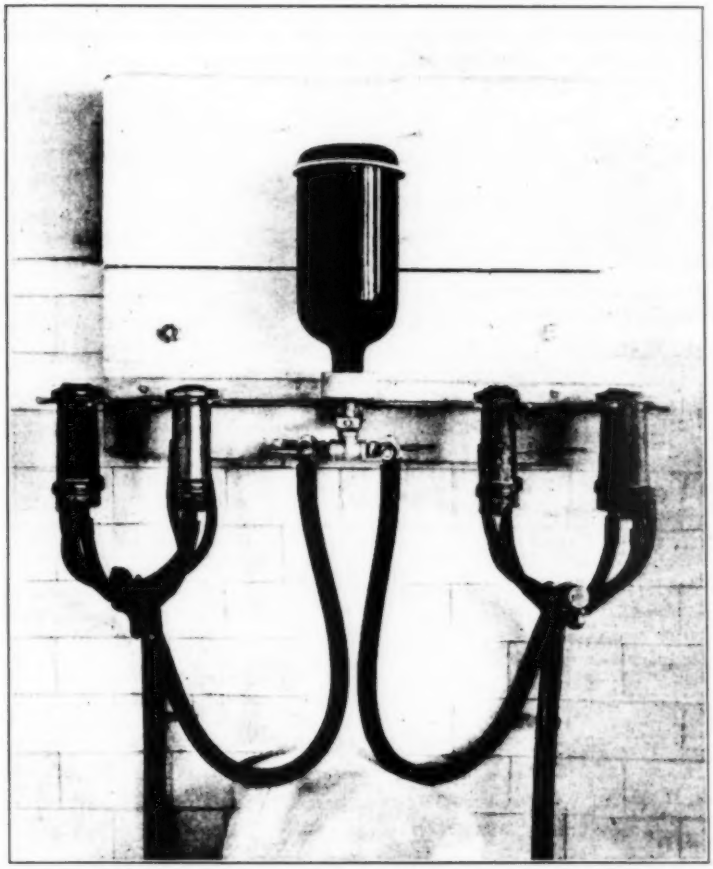

FIG. 2. HOME-MADE CHLORINE OR SODIUM HYDROXIDE STERILIZATION RACK.
A brown glass bottle should be used for chlorine solution in order to protect it from light.

The sodium hydroxide (lye) solutions were prepared by dissolving sodium hydroxide flakes in water to make solutions with strengths of 0.3 and 0.5 per cent. The sterilizer rack used was the standard one recommended for chemical sterilization of rubber parts. The results from the use of chlorine and of sodium hydroxide appear in table 2.

Results.—The treatment of milking machine rubber parts with chlorine of a strength of 200 parts per million, with sodium hydroxide 0.3 per cent solution, each of which was used in the sterilizing rack, and with sodium hydroxide 0.5 per cent solution used in an earthen crock, showed that the 0.3 per cent sodium hydroxide affected the life of rubber parts the same as

TABLE 2

The effect of chlorine and lye sterilization on the life of rubber parts of milking machines
(Machines were used three times per day)

	CHLORINE 200 P.P.M. USED IN STERILIZING RACK (CHANGED DAILY)	NaOH 0.3 PER CENT USED IN STERILIZING RACK (CHANGED DAILY)	NaOH 0.5 PER CENT USED IN EARTHEN CROCK (CHANGED WEEKLY)
Teat cup liners			
Life in weeks	11	11	10
Number of sterilizations	231	231	210
Number of cow-hours operated	2,772	2,772	2,520
Number of sets of rubbers used in determining average life	8	4	4
Short milk tubes			
Life in weeks	14		
Number of sterilizations	294		
Number of cow-hours operated	3,528		
Number of sets of rubbers used in determining average life	6		
Short air tubes			
Life in weeks	31		
Number of sterilizations	651		
Number of cow-hours operated	7,912		
Number of sets of rubbers used in determining average life	2		

did chlorine of 200 parts per million; that is, the teat cup liners gave 11 weeks of service. With the use of sodium hydroxide 0.5 per cent solution, the life of the rubbers was 10 weeks.

The results reported in table 2 were secured from three-times-a-day milkings, and the average life of the tea cup liners totaled 2,772 cow-hours of operation. The use of chlorine, as reported in table 1 in which the teat cup liners lasted 12 weeks, was based on twice-a-day milkings and a total of 1,092 cow-hours of service. Evidently, therefore, the life of the teat cup liners is affected more by the number of weeks of service than by the number of cow-hours of operation. The same results were secured with chlorine sterilization of the short milk tubes and the short air tubes.

EFFECT OF HEAT AND CHEMICAL STERILIZATION ON THE BACTERIA COUNT OF RUBBERS

Procedure.—The bacteria count of the milking machine rubbers was obtained by rinsing the inner surfaces of the rubbers with sterile distilled water and then plating the water. The rinsings were carried out in the

following manner: The teat cups and long milk tubes were attached to the claws, and the assembled units were suspended from the sterilizing rack (figure 2). A sterile cork was inserted in the open end of the long milk tube. One hundred cc. of sterile water was then poured into the teat cups, and the assembled rubbers were shaken in such a way that the water came in contact with all parts of the inner surface of the rubbers. The water removed from the tubes was then plated with standard agar media. In certain instances media made of 15 grams of agar and 20 cc. of skimmilk per liter were used. Incubation of the plates was at 37° C. and 60° C. The higher temperature and the special media were used to detect the presence of thermophilic bacteria.

Results.—The results of the bacteria determinations appear in table 3. They show that of the heat sterilization methods used, the most satisfactory was the procedure of heating to 185° F., allowing the rubbers to remain in the water for 20 minutes, and then draining the water from the tank.

TABLE 3

Bacteria counts of sterile rinse water passed through assembled rubber parts

TREATMENT OF RUBBERS	MEDIA AND INCUBATION TEMPERATURES	AVERAGE NUMBER OF BACTERIA PER CC. OF RINSE WATER	NUMBER OF SAMPLES PLATED
	Heat sterilization		
170° F. for 20 minutes left in water water between milkings	Standard 37° C.	125	96
	Standard 60° C.	746	68
	*Special 60° C.	6,430	26
170° F. for 20 minutes. Water then drained from heating tank	Standard 37° C.	11	10
	Standard 60° C.	4,420	10
	Special 60° C.	350	10
185° F. No more heat added, tank drained after 20 minutes	Standard 37° C.	3	14
	Standard 60° C.	0	14
	Special 60° C.	0	14
	Chemical sterilization		
Chlorine 200 p.p.m. used in sterilizing rack	Standard 37° C.	45†	114
	Standard 60° C.	0	52
0.3 per cent NaOH solution used in sterilizing rack	Standard 37° C.	372	16
	Standard 60° C.	0	16
0.5 per cent NaOH solution in crock, new solution used once per week	Standard 37° C.	148	18
	Standard 60° C.	0	18

* Special media for determining the presence of thermophiles consisted of 15 grams of agar and 20 cc. of skimmilk per liter.

† Three high counts that occurred in warm weather in July are not included in this average.

Table 1 shows that this method does not shorten the life of the rubbers significantly when compared with heating at 170° F. for 20 minutes. The higher temperature also destroys the thermophilic bacteria, which appear to survive the lower temperature.

When chlorine and sodium hydroxide were used, thermophilic bacteria did not develop, and the total number of bacteria remained within reasonable limits. Chlorine sterilization gave three very high counts during the summer period when it is difficult to maintain the strength of the solution. In the hands of the average dairyman, the use of chlorine is less satisfactory because the rubbers must be free from organic material and the strength of the solution regularly checked, if the germicidal quality is to be assured.

CONCLUSIONS

1. Heat sterilization of milking machine rubber parts, using water at a temperature of 185° F. and leaving the rubbers in the water for 20 minutes, was the most satisfactory method of heat sterilization employed. The life of the rubber parts was not materially reduced by this method when compared with heating at 170° F. for 20 minutes.

2. When the rubbers were heated at a temperature of 170° F. and held at that temperature for 20 minutes, or when they were left in the water to cool gradually until next milking time, there was evidence that thermophilic bacteria developed.

3. Chlorine in the solution of 200 parts per million, or sodium hydroxide solution of a strength of 0.3 and 0.5 per cent, gave bacteria counts somewhat higher than those resulting from heat sterilization at 185° F. They were, however, effective in controlling thermophilic bacteria. The life of the rubbers with chlorine and with sodium hydroxide sterilization was approximately the same.

4. The life of the teat cup liners resulting from each of the different methods of heat sterilization was approximately the same but was 33 per cent shorter than when chlorine of a strength of 200 parts per million was used.

5. In chlorine sterilization, the length of service had more influence in reducing the life of the rubbers than did cow-hours of operation.

IRRADIATED MILK: THE INFLUENCE OF FAT CONTENT AND TIME OF EXPOSURE ON THE ANTIRACHITIC POTENCY

G. C. SUPPLEE, G. E. FLANIGAN, AND R. C. BENDER
The Dry Milk Company Research Laboratories, Bainbridge, New York
AND
M. J. DORCAS
The National Carbon Company, Cleveland, Ohio

Studies recently reported (1) show that a large percentage of the incident ultraviolet rays within the antirachitic range are absorbed by milk films less than 0.10 millimeter thick. Notwithstanding this high percentage absorption by the immediate surface layers, a relatively high antirachitic activity was obtained in less than 2 seconds and in some instances in less than 1 second, in rapidly flowing films. The data also revealed that the particular conditions of treatment involving the application of radiations of high intensity to films of known thickness and rate of flow, imparted measurable antirachitic properties to milk derivatives containing little or no butter fat. The degree of activation obtained in milks of variable fat content was not in proportion to the amount of fat present. It has been shown by Hess and coworkers (2) (3) (4) that clinical, as well as the laboratory assay, results from milks containing 1.2 per cent butter fat and 3.6 per cent butter fat were substantially the same when identical methods of irradiation were used. In view of the laboratory and clinical evidence, it has seemed desirable to obtain further information regarding the antirachitic activation as influenced by the character and thickness of the milk film, the fat content and time of exposure.

EXPERIMENTAL

The experimental procedures employed were the same as those already reported, wherein a carbon arc of the same character and intensity of the energy output was used for irradiating milk films on the especially devised flow board which permitted the determination of the film thickness, speed of flow and volume delivered by the film per unit of time (1). The biological data were obtained in the usual manner and the results (Table 1) reduced to the same comparable basis previously used (1) (5) (6).

The results indicate that the maximum antirachitic activity of milk resulting from direct irradiation does not parallel the fat content. It appears, however, that the fat content does influence to a certain but limited degree the rate at which the antirachitic properties are imparted to the milk. Other conditions being the same during the irradiation of films 0.02

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TABLE 1

The Vitamin D concentration of milk as affected by film thickness, film capacity, fat content and amount of applied energy (2000–3000 A°)

SAMPLE	TEST SUBSTANCE	MILK FILM CHARACTERISTICS			EXPOSURE PERIOD	TOTAL ERG/ PER CC. ($\times 10^9$)	TOTAL QUANTA PER CC. ($\times 10^{14}$)	TOTAL MILK FED	VITAMIN D PER CC. ($\times 10^{15}$)	VITAMIN D PER MG. OF FAT ($\times 10^{18}$)
		Capacity per inch per minute	Thickness	Vertical distance of travel during exposure						
		gms.	*mm.*	*cm.*	*secs.*			*cc.*	*mols.*	*mols.*
1 SM	Skimmed Milk	2.12	0.02	0.66	1	8,945	11,851	20	.....	.....
2 SM	" "	"	"	10.79	16	143,120	179,610	50	90	300.0
3 SM	" "	"	"	21.61	32	286,240	359,220	40	112	373.0
4 SM	" "	"	"	48.64	72	644,060	853,272	40	112	373.0
1–06	Milk, 0.6% Fat	2.12	0.02	0.66	1	8,945	11,851	60	75	12.5
2–06	" " "	"	"	5.38	8	71,560	89,805	30	150	25.0
3–06	" " "	"	"	10.79	16	143,120	179,610	30	150	25.0
4–06	" " "	"	"	48.64	72	644,060	853,272	30	150	25.0
5–06	" " "	34.02	0.11	40.64	1.87	3,769	4,994	60	75	12.5
1–12	Milk, 1.2% Fat	2.12	0.02	0.66	1	8,945	11,851	60	75	6.25
2–12	" " "	"	"	5.38	8	71,560	89,805	30	150	12.50
3–12	" " "	"	"	10.79	16	143,120	179,610	20	225	18.75
4–12	" " "	"	"	48.64	72	644,060	853,272	20	225	18.75
5–12	" " "	34.02	0.11	40.64	1.87	3,769	4,994	20	225	18.75
6–12	" " "	192.78	0.23	40.64	0.89	972	1,288	50	90	7.50
1–36	Milk, 3.6% Fat	2.12	0.02	0.66	1	8,945	11,851	30	150	4.16
2–36	" " "	"	"	5.38	8	71,560	89,805	15	300	8.32
3–36	" " "	"	"	10.79	16	143,120	179,610	15	300	8.32
4–36	" " "	"	"	48.64	72	644,060	853,272	15	300	8.32
5–36	" " "	8.78	0.10	5.08	1	2,236	2,692	20	225	6.25
6–36	" " "	"	"	9.52	1.87	4,181	5,538	15	300	8.32
7–36	" " "	"	"	40.64	8	17,888	23,696	15	300	8.32

TABLE 1—(*Continued*)

The Vitamin D concentration of milk as affected by film thickness, film capacity, fat content and amount of applied energy (2000–3000 A°)

SAMPLE	TEST SUBSTANCE	MILK FILM CHARACTERISTICS			EXPOSURE PERIOD	TOTAL ERGS. PER CC. ($\times 10^9$)	TOTAL QUANTA PER CC. ($\times 10^{14}$)	TOTAL MILK FED	VITAMIN D PER CC. ($\times 10^{10}$)	VITAMIN D PER MG. OF FAT ($\times 10^{10}$)
		Capacity per inch per minute	Thickness	Vertical distance of travel during exposure						
		gms.	*gms.*	*cm.*	*secs.*			*cc.*	*mols.*	*mols.*
8–36	Milk, 3.6% Fat	34.02	0.11	21.60	1	2,016	2,671	30	150	4.16
9–36	" " "	"	"	40.64	1.87	3,769	4,994	20	225	6.25
10–36	" " "	102.06	0.23	33.02	1	976	1,293	35	128	3.55
11–36	" " "	"	"	40.64	1.24	1,210	1,603	25	180	5.00
12–36	" " "	"	"	61.72	1.87	1,825	2,418	15	300	8.32
13–36	" " "	192.78	0.23	40.64	0.89	972	1,288	35	128	3.55
14–36	" " "	"	"	76.20	1.87	2,041	2,704	25	180	5.00
15–36	" " "	272.16	0.46	40.64	0.89	486	644	37	121	3.36
16–36	" " "	"	"	76.20	1.87	1,020	1,352	30	150	4.16
17–36	" " "	442.26	0.50	40.64	0.60	447	592	40	112	3.11
18–36	" " "	"	"	66.80	1	742	989	40	112	3.11
19–36	" " "	"	"	125.00	1.87	1,394	1,847	25	180	5.00
1–72	Milk, 7.2% Fat	2.12	0.02	0.66	1	8,945	11,851	17.5	257	3.56
2–72	" " "	"	"	5.38	8	71,560	89,805	17.5	257	3.56
3–72	" " "	"	"	10.79	16	143,120	179,610	17.5	257	3.56
4–72	" " "	"	"	48.64	72	644,060	179,610	17.5	257	3.56
5–72	" " "	34.02	0.11	40.64	1.87	3,769	4,994	17.5	257	3.56

millimeter thick a higher antirachitic potency is produced in milks containing 3.6 per cent fat and 7.2 per cent fat during the first momentary exposure (about 1 second) than is produced in milks of lower fat content. When the milks of low fat content irradiated in the thinnest obtainable films are subjected to longer exposure periods, the antirachitic property is increased during a period of about 16 seconds; the period during which there is an increase being the longest for the milk containing the least amount of fat. The period during which there is an increase in the potency of milk containing 3.6 per cent fat (whole milk) appeared to be limited to about 8 seconds, under similar conditions of treatment. When milk containing 7.2 per cent butter fat is similarly treated there appears to be no increase in potency after the first second of exposure. The period during which the antirachitic potency of whole milk develops most rapidly when irradiated in thicker and more rapidly flowing films, appears not to exceed about 2 seconds. This seems to again confirm the conclusion that vitamin D synthesis in milk during direct irradiation is substantially instantaneous in the immediate surface layer. The homogenization of whole milk prior to irradiation, whereby the surface area of a unit amount of fat exposed to the milk serum is greatly increased, does not permit any significantly greater degree of activation, or increased rate of activation, under the conditions used, than does the unhomogenized milk.

The data presented in this paper clearly indicate that substances contained in milk other than the milk fat are important factors which determine its antirachitic properties during irradiation. It is quite probable that a further investigation of such substances will explain wholly, or in part, the merits of irradiated milk as shown by the clinical investigations of Hess and Lewis (3) (4), and others. The rapidity of vitamin D synthesis during momentary exposure periods and the failure to induce a further significant increase in potency during longer exposure periods, raises certain questions regarding the mechanics of activation. A full explanation of the results may necessitate the consideration of many factors concerning the substance or substances affected by radiant energy. Notwithstanding the possible influence of the chemical constitution and physical relationship of such substances as they exist in milk, the observations of Bourdillon *et al.* (7), Reerink and Van Wijk (8), Marshall and Knudson (9) and Windaus *et al.* (10), are, no doubt, pertinent, in certain respects at least, to the results recorded herein.

CONCLUSIONS

1. The fat content influences to a certain but limited degree the rate at which antirachitic properties are produced in milk by direct irradiation with ultraviolet rays.

2. Milks containing little or no butter fat may be activated to a substan-

tial degree, but the degree of potency ultimately obtained is not reached as quickly as in those milks containing larger amounts of fat. The degree of potency attainable in milk with the larger amounts of fat does not increase in proportion to the amount of fat present.

3. Milks containing normal amounts of fat may be activated substantially to their maximum degree by a momentary exposure of less than 2 seconds, if suitable intensity of ultraviolet radiations are applied to films of suitable thickness and flow characteristics.

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REFERENCES

(1) SUPPLEE, G. C., AND DORCAS, M. J. Irradiated milk: The transmission and antirachitic activation of milk films by ultra-violet radiations. JOUR. DAIRY SCIENCE 17: 433. 1934.

(2) SUPPLEE, G. C., DORCAS, M. J., AND HESS, A. F. Irradiated milk: The energy requirements for antirachitic activation. Jour. Biol. Chem. 94: 749. 1932.

(3) HESS, A. F., AND LEWIS, J. M. Milk irradiated by the carbon arc lamp: A clinical and laboratory study of rickets. Jour. Am. Med. Assoc. 99: 647. 1932.

(4) HESS, A. F., AND LEWIS, J. M. An appraisal of antirachitics in terms of rat and clinical units. Jour. Am. Med. Assoc. 101: 181. 1933.

(5) SUPPLEE, G. C., HANFORD, Z. M., DORCAS, M. J., AND BECK, H. H. Irradiated milk: The amount of vitamin D and its rate of formation. Jour. Biol. Chem. 95: 687. 1932.

(6) SUPPLEE, G. C., BECK, H. H., AND DORCAS, M. J. Irradiated milk: The influence of the intensity and character of the radiations on the antirachitic potency. Jour. Biol. Chem. 98: 769. 1932.

(7) BOURDILLON, R. B., FISCHMANN, C., JENKINS, R. G. C., AND WEBSTER, T. A. Absorption spectrum of vitamin D. Proc. Roy. Soc. London, Series B, 104: 561. 1929.

(8) REERINK, E. H., AND VAN WIJK, A. The vitamin D problem: The photochemical reactions of ergosterol. Biochem. Jour. 23: 1294. 1929.

(9) MARSHALL, A. L., AND KNUDSON, A. The formation of vitamin D by monochromatic light. Jour. Am. Chem. Soc. 52: 2304. 1930.

(10) WINDAUS, A., AND LUTTRINGHAUS, A. Uber das bestrahlte Ergosterin. Deut. Med. Woch. 58: 1669. 1932.

THE EFFECT OF PASTEURIZATION UPON THE VITAMIN C CONTENT OF MILK

C. G. KING AND W. A. WAUGH

Department of Chemistry, University of Pittsburgh, Pittsburgh, Pa.

The protection from bacterial infections which is afforded by pasteurization of municipal milk supplies is an acknowledged factor of great importance. In general those who are actively working in the field of dairy science, particularly in relation to public health, are agreed that the advantages of proper pasteurization greatly outweigh the possible minor disadvantages (1). The further elimination of any apparent disadvantages represents important progress insofar as it tends (a) to increase the practice of proper pasteurization, or, (b) to improve the quality and quantity of milk which is consumed.

Of the recognized nutritive factors in milk, vitamin C is probably the most sensitive to destruction, due primarily to its oxidation. Such oxidative changes are greatly accelerated by exposure to air at high temperatures and by the presence of metallic catalysts, particularly copper (2) (5). Hence a quantitative study of the effect of pasteurization upon the vitamin C content of milk should provide an extremely sensitive index to possible changes in its chemical nature or its nutritive value.

The present investigation has included a quantitative study of the vitamin C content of representative western Pennsylvania market milk as received at the pasteurizing plant, and the effect of pasteurization by the (a) Electropure, (b) StamVik, and (c) holding processes, each of the three units receiving milk from the same agitated raw milk tank. The primary purpose of the investigation was to find whether there was a significant amount of vitamin C destroyed during the Electropure or StamVik processes of pasteurization.

The dairy herds supplying milk were chiefly on summer pasture, supplemented with varying amounts of hay and grain.

PASTEURIZING EQUIPMENT

The Electropure pasteurizer (3) (Figure 1) was a small standard commercial unit (capacity 100 gal. per hour), equipped throughout with aluminum piping and fittings. The upper part of the cooler served as a heat exchanger, preheating the milk to approximately 125° F., after which it passed through a filter and thence to the Electropure heating unit where the temperature was raised to 161–3° F. by the resistance of the upward-flowing column of milk (between carbon electrodes, 220 V. A.C.). Having

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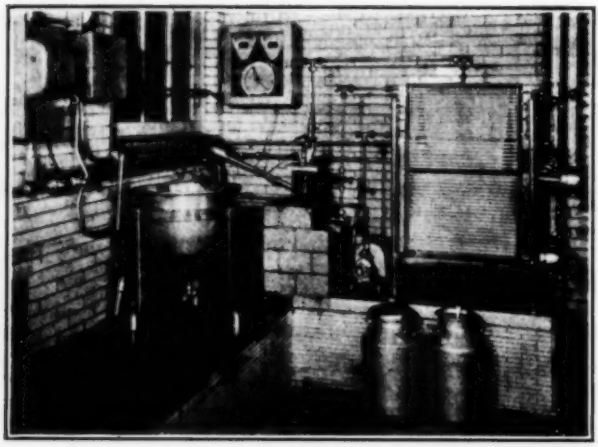

FIG. 1

reached the pasteurizing temperature, there was a time interval of 16 seconds before the milk reached the top of the cooler, the rate of flow being controlled automatically by the speed of a pump which was in turn regulated by the temperature of the milk leaving the heating chamber. The lower section of the cooler was supplied with brine to complete the cooling.

The StamVik pasteurizer (Figure 2) was a standard commercial unit (4) (capacity 500 gal. per hour), also equipped throughout with aluminum piping and fittings. The cooler-regenerator and filter equipment was of the same type as that in the Electropure unit (entirely aluminum). The

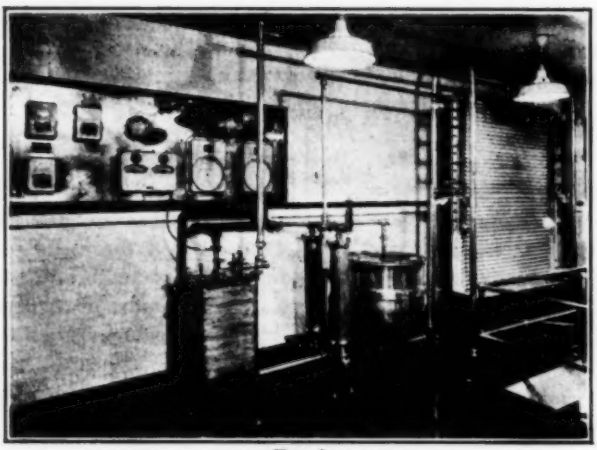

FIG. 2

essential characteristic of the StamVik process is the heating unit, constructed of a series of hollow aluminum plates, with counter current heating of the milk by means of hot water flowing inside the aluminum plates (4).

The time interval for reaching the pasteurizing temperature in the heating unit (161–3° F.) is only 17 seconds, in which respect it approaches the Electropure process, where the corresponding interval is approximately 4 seconds for the unit used.

During the greater part of the experimental period, pasteurization by the holding method was carried out in laboratory-scale equipment. During the latter part of the study a commercial pasteurizing vat became available (150 gal. capacity), which afforded an opportunity of verifying the results obtained on a laboratory scale. Raw milk was drawn from the same tank which supplied the other units into a new, heavily-tinned covered milk can which was placed in a large vat of hot water adjusted to raise the milk to pasteurizing temperature (143–145° F.) in five to ten minutes, with slow agitation. As soon as 142° F. was reached, the vat of water was cooled to 143–5° and held at that temperature for 30 minutes. The milk was then cooled quickly over a brine cooler (identical with that used for the milk from the other processes) from which bottles were filled and capped and placed in refrigeration (40–50° F.) until used.

During the latter part of the period, normal plant practice was followed in pasteurizing for 30 minutes in a commercial covered, tin-lined vat at 143–5° F. with normal agitation during heating by a rotating hot water coil, after which agitation was stopped until the pasteurization was completed. It was then agitated slowly as it flowed to the cooler, the bottled samples being taken from the bottom of the cooler after about five minutes.

Samples of raw milk were taken from the same supply used for pasteurizing by the three different methods, and all bottled samples (3 of each per day) were placed in a crate together for refrigeration until delivered to the laboratory the same day or the following morning.

Plant operations were carried out by workmen in the dairy who made every effort to keep the products as nearly comparable in every way as was possible under plant conditions.

ANIMAL ASSAYS

Young male guinea-pigs were purchased whose average weight was about 300 grams. They were placed in individual cages and fed a modified Sherman diet (supplemented by cod-liver oil) as a basal vitamin C-free ration. The only modification in the basal ration was to decrease the amount of milk solids equivalent to 30 cc. of fresh whole milk per day, to compensate for the additional supply of milk in test feeding. During a preliminary period of 7 to 10 days, the animals were fed a supplement of fresh spinach and young, crisp carrots, to assure that they were growing

normally, in apparent good health, and in a good state of nutrition at the beginning of the test period. Each animal was given 10 cc. of milk for two days before the test period began, to accustom it to drinking from a pipette.

From the beginning of the assay period, the animals received only their basal diet and water, supplemented by the 30 cc. of milk being tested, fed directly from a pipette. The results of the test are given in table 1.

TABLE 1

Feeding test with 30 cc. of milk as the only antiscorbutic

TYPE OF MILK FED	NO. OF ANIMALS	WEIGHT IN GRAMS	NO. ANIMALS SURVIVING 56-DAY TEST	AVER. SURVIVAL OF THOSE DYING BEFORE 56 DAYS	AVERAGE SCURVY SCORE AT AUTOPSY
Raw	8	327	3	40	7.1
Vat Pasteurized	8	325	0	27	14.8
Electropure "	8	332	2	44	7.0
StamVik "	8	327	1	42	8.0

Realizing that general market milk would be somewhat low in vitamin C value, we were reasonably sure that 30 cc. per day would not be adequate for complete protection (5). Feeding larger quantities, up to the amount required for full protection, *e.g.*, 60 to 80 cc., involves keeping the animals on practically a whole-milk diet, which is not suitable for animals accustomed to a diet high in roughage. Hence we decided to use one series of animals receiving only the 30 cc. of milk supplement, and another receiving milk and an additional supplement of orange juice. The former furnished a basis for direct comparison based upon survival and weight change, and the latter served to raise the total vitamin intake to a level which permitted better survival and growth, and consequently a more satisfactory basis for evaluating small differences. Each group received exactly the same supplement of orange juice.

The animals receiving orange juice were fed carefully from graduated pipettes, so that all received exactly the same amount—the quantity being gauged to keep most of the animals from losing weight or developing severe scurvy. The milk supplement was raised from 15 to 30 cc. for these groups on the 28th day. In earlier studies with the pure vitamin (6) it has been found that a daily intake of 0.5 mg. per day provides an excellent level for quantitative interpretation. Approximately such a level was provided by feeding 15 to 30 cc. of milk and 0.5 to 1.0 cc. of orange juice daily. For assaying and comparing foods which are relatively low in vitamin C, we believe such a procedure has a distinct advantage, in that it (a) permits the animals to receive a more nearly normal dietary, (b) supplies a vitamin level most sensitive to assay differences, and (c) provides a marked economy in feeding time. Table 2 furnishes a summary of the results obtained.

TABLE 2
Feeding test, milk supplemented by 1 to 2 cc. of orange juice

TYPE OF MILK FED	NO. OF ANIMALS	WEIGHT IN GRAMS	NO. ANIMALS SURVIVING 56-DAY TEST	AVER. SURVIVAL OF THOSE DYING BEFORE 56 DAYS	AVERAGE SCURVY SCORE AT AUTOPSY
Raw	7	290	2	37	1.8
Vat Pasteurized	7	296	7	.....	7.0
Electropure "	7	314	7	.....	1.4
StamVik "	7	317	5	.....	1.8

TITRATION OF VITAMIN C

The oxidation-reduction indicator, 2, 6-dichlorophenolindophenol, introduced by Mansfield Clark and associates (7), was first adapted for vitamin C titration by Tillmans and associates (8). With reasonable reservation in considering possible interfering substances, we have found the method to be applicable for studying most plant and animal tissues. Details to be followed in using the indicator and data obtained by its use have been given in another paper (9). The titration of vitamin C in milk proved to be relatively satisfactory in the presence of trichloroacetic acid and furnished data in good agreement with the animal feeding tests, as shown by the summary of the titrations given in table 3.

TABLE 3
Direct titration of vitamin C in milk

TYPE OF MILK	APPROXIMATE TITRATION VALUE MG. VITAMIN PER 10 CC.
Raw	0.10
Vat Pasteurized	0.04
Electropure Pasteurized	0.10
StamVik Pasteurized	0.10

DISCUSSION OF RESULTS

It has been shown in previous investigations (3) (5) that prolonged heating of milk exposed to air tends to destroy vitamin C more severely than heating to the boiling point for a short time. In the Electropure and StamVik processes there is only a very short interval for preheating and pasteurizing (16 seconds after reaching 162° F.) during which time the milk is protected from air. The time of exposure as the milk flows over the surface cooler is approximately 6 seconds. This, together with the use of aluminum equipment probably accounts for the finding that there was no significant loss in vitamin C as a result of pasteurization by either process.

The same conclusion is even more definitely indicated by the direct chemical titration data.

Although the animal assay of milk pasteurized by the holding method included that from a commercial unit and in part that from a laboratory unit, there was no evident change in the animal response when the type of pasteurizer was changed, and it is believed that there was no significant difference in the results with laboratory and commercial equipment. Frequent titrations of the two products with the dye-indicator would have demonstrated even smaller differences that could be detected by animal assays. A further study of vitamin C destruction by other pasteurization methods and with different types of equipment will be reported in the near future.

The degree of protection from scurvy afforded by 30 cc. of raw milk is in good agreement with previous data on the vitamin content of cow's milk and also in good agreement with the data obtained by direct chemical titration.

The destruction of a significant portion of the vitamin C content by slow pasteurization is accounted for by the prolonged opportunity for oxidation, resulting in part from exposure to air and perhaps in part by a greater exposure to metals, especially where small quantities of copper may be picked up from well-tinned commercial equipment such as used in the present study.

In view of the relatively rapid depletion of vitamin C from the body tissues when the diet is deficient, and in view of the fact that considerable numbers of children and infants receive only haphazard supplies of supplementary antiscorbutic foods when on a high-milk diet, it is an item of importance to conserve the vitamin content naturally provided. Unfortunately, a large portion of our population does not yet realize the need for providing special antiscorbutic foods to very young children deprived of mother's milk. Although clinical survey is not considered common in America, it does occur occasionally, and there is good evidence to indicate that vitamin deficiencies are significant in relation to health at levels far above those characterized by the so-called deficiency diseases (5) (9) (10). Dalldorf has reported finding an incidence of "latent scurvy" revealed by weakened blood capillaries, reaching 35 to 66 per cent of the children in poor families in New York (11).

The poorer survival on an equal dietary level shown by the animals receiving raw milk was apparently due entirely to the higher incidence of infections in this group. The experimental work was carried out during July and August when the bacterial counts were relatively high. The animals receiving pasteurized milk had an advantage in being protected from infectious types of organisms almost certain to be present in a general raw milk supply. Such biological factors serve to emphasize the value of

knowing the chemical nature of the vitamins and of having available chemical methods of estimation. The scurvy scores of the animals furnish a fairly specific indication of their nutritive condition, however, so that the above interpretation of vitamin C intake on the different types of milk was not misinterpreted by variations in survival alone.

The authors believe that the above experimental record gives strong evidence against the suggestion occasionally made that pasteurization necessarily brings about a significant impairment in the nutritive value of milk. It is unlikely that there is any essential nutritive factor present which is less stable than vitamin C, but there is constantly accumulating evidence for the need of protection from infections (12).

SUMMARY

There is no significant destruction of vitamin C in milk by the Electropure (electrical conductivity) or StamVik (flash contact) methods of pasteurization when all-aluminum equipment is used. This is probably due to: (a) a very short heating time, (b) methods of heating, (c) protection from atmospheric oxidation during heating, and (d) a minimum exposure to metals which catalyse oxidation.

The finding is based upon two series of animal assays of the raw and pasteurized milk and also upon titration of the vitamin by means of 2, 6-dichlorophenolindophenol.

Pasteurization of milk from the same tank either on a laboratory scale or in a commercial vat at 143–5° for 30 min. resulted in a significant destruction of vitamin C.

The importance of conserving the natural vitamin C content of milk during pasteurization has been indicated.

REFERENCES

(1) Park, W. H., and Holt, L. E. Med. News 83: 1066. 1903.
Zinsser, H., and Russell, F. F. Bacteriology, Chap. 53. (D. Appleton & Co., N. Y.). 1925.
Rogers, L. A. The fundamentals of dairy science, Chap. 10 and 14. (Chemical Cat. Co., N. Y.). 1928.
Hess, A. F., and Unger, L. J. Am. J. Dis. Children 17: 221. 1919.
(2) Hess, A. F., and Weinstock, M. J. Am. Med. Assoc. 82: 952. 1924.
Anderson, E., Dutcher, R. A., Eckles, C. H., and Wilbur, J. W. Science 53: 446. 1921.
Schwartze, E. W., Murphy, F. J., and Cox, G. J. J. Nutrition 4: 211. 1931.
(3) King, C. G. Trans. Am. Electrochem. Soc. 62: 233. 1932.
(4) StamsVik, A. U. S. Pat. No. 1,916,395 (July 4, 1922) and No. 1,918,434 (July 18, 1933).
(5) Sherman, H. C., and Smith, S. L. The vitamins, Chap. 4. (Chem. Cat. Co., N. Y.). 1931.
Sherman, H. C. Am. J. Public Health 23: 1031. 1933.

(6) WAUGH, W. A., AND KING, C. G. Science 75: 357, 360. 1932; J. Biol. Chem. 97: 325. 1932.
WAUGH, W. A., BESSEY, O. A., AND KING, C. G. Trans. Am. Soc. Exptl. Biol. Med. 30: 1281. 1933.
(7) COHEN, B., GIBBS, H. D., AND CLARK, W. M. Pub. Health. Rep., U. S. P. H. S., 39: 804. 1924; 40: 649. 1925.
(8) TILLMANNS, J., HIRSCH, P., AND HIRSCH, W. Z. Untersuch. Lebensmittel 63: 1. 1932.
(9) BESSEY, O. A., AND KING, C. G. J. Biol. Chem. 103: 687. 1933.
(10) SHERMAN, H. C. Chemistry of food and nutrition, Chap. 24. (Macmillan Co., N. Y.). 1932.
SURE, B. The Vitamins, Chap. 4 and 9. (Williams and Wilkins, Balt.). 1933.
MCCOLLUM, E. V., AND SIMMONDS, N. The newer knowledge of nutrition, p. 506. (Macmillan Co., N. Y.). 1929.
(11) DALLDORF, G. Am. J. Dis. Children 46: 794. 1933.
(12) BIGELOW, G. H., AND FEEMSTER, R. F. Am. J. Pub. Health 23: 571. 1933.
BIGELOW, G. H., AND FORSBECK, F. C. Am. J. Pub. Health 23: 1094. 1933.
BROOKS, P. B. Am. J. Pub. Health 23: 1165. 1933.
HUCKER, G. J. N. Y. Agr. Expt. Sta., Tech Bul. No. 199. 1933.

RATE OF GROWTH AND ACID PRODUCTION OF *STREPTOCOCCUS LACTIS*

J. M. SHERMAN AND H. M. HODGE
Cornell University, Ithaca, New York

Evidence has been published elsewhere which indicates that slow growth enables an organism to better adapt itself to its environment and hence to exhibit greater viability when exposed to deleterious environmental factors. It has also been pointed out that among bacteria and higher organisms those species which have become adapted to growth under conditions which are unfavorable for most forms of life have relatively slow rates of growth.[1]

If this hypothesis is correct, it is logical to expect that with any given organism the more slowly growing strains should show greater tolerance for the environmental impediments with which it has had to contend during its course of evolution. In the case of *Streptococcus lactis*, acidity is the most obvious limitation to growth under the only natural conditions we know it—in milk and milk products. With this thought in mind, we have attempted to learn whether or not there does exist a relationship between rate of growth and total acid producing power (acid tolerance) in *Streptococcus lactis*.

While we do not wish to draw sweeping conclusions from the small body of data contained in this note, it is presented for what it may be worth as a contribution to the general problem and, also, because it is barely possible that this line of approach might yield useful results in the selection and development of strains of *Streptococcus lactis* for certain dairy purposes.

An obvious way to test the theory would be to isolate a number of cultures of *Streptococcus lactis* and determine the rate of growth and acid producing power of each. This procedure was not considered sound, and was not attempted, because in a given pure culture there may be slow and rapidly growing strains, one of which would give the culture the characteristic of fast growth and the other that of acid tolerance. (However, it may be mentioned incidentally that of the two cultures used in this study the one which grew more slowly had the ability to produce the greater amount of acid.) It is apparent that in any given culture, unless very old, the fastest growing strains would be present in greatest numbers, while the strains which have a slower rate of reproduction would naturally occur in smaller numbers. With this fact in mind it was reasoned that, if our working hypothesis is correct, two things should be demonstratable:

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[1] Sherman, J. M., and Cameron, G. M. Jour. Bact. 27: 23. (Abstract. Data in full not yet published.) 1934.

(1) If a series of flasks of sterile milk were inoculated from a milk culture of *Streptococcus lactis* in amounts ranging from say 1 cc. to 10^{-8} cc., a greater amount of acid should be produced in those receiving the larger inocula. The reason for expecting this result is that in the high dilutions of the original culture there would be present only the most rapidly growing strains which, according to the hypothesis under test, could not endure such a high degree of acidity.

(2) If a culture of *Streptococcus lactis* were grown in milk with very frequent transfer for a period of time, the resulting culture should have a lower acid producing power than the original culture; and, further, contrary to the above case, the amounts of acid produced from varying amounts of inocula should be more nearly the same. The reason for expecting these results is simply that with frequent transfers the slow growing strains would be "weeded out" and left behind so that the resulting culture would contain only fast growing organisms.

A laboratory stock culture of *Streptococcus lactis* (No. 8) 24 hours old in litmus milk was inoculated into flasks of sterile skimmed milk, the inocula varying in amount from 1 cc. to 10^{-8} cc. These flasks, sealed with sterile rubber stoppers, were incubated at 37° C. for 7 days and then titrated for total acidity. Quadruplicate flasks were inoculated with each dilution. The original culture was then carried in litmus milk at laboratory temperature with 12-hour subculturing for ten transfers; this being done in test tubes containing about ten cc. of milk and transfers made with an ordinary inoculating loop. At the end of this period, flasks of sterile skimmed milk were inoculated with this culture for acid production as in the case of the parent culture. The results obtained are given in table 1.

An inspection of these data shows that the predicted results were obtained: (1) The amount of acid produced by the original culture decreased with the size of the inoculum. (2) A similar variation in acid production

TABLE 1

Acid Production by Varying Amounts of Streptococcus lactis (No. 8)

INOCULUM (CC.)	PER CENT ACID AS LACTIC ACID	
	24-hour Stock Culture	Serial 12-hour Culture
1	0.82	0.71
10^{-1}	0.76	0.71
10^{-2}	0.75	0.70
10^{-3}	0.76	0.69
10^{-4}	0.75	0.68
10^{-5}	0.76	0.70
10^{-6}	0.76	0.69
10^{-7}	0.78	0.68
10^{-8}	0.72	No growth

with size of inocula did not occur in the serial 12-hour culture; nor was the amount of acid produced from the 1 cc. inoculum as great as in the case of the original culture.

The experiment was repeated using another strain of *Streptococcus lactis* (No. 21) with similar results (table 2).

TABLE 2

Acid Production by Varying Amounts of Streptococcus lactis (No. 21)

INOCULUM (CC.)	PER CENT ACID AS LACTIC ACID	
	24-hour Stock Culture	Serial 12-hour Culture
1	0.71	0.63
10^{-1}	0.67	0.62
10^{-2}	0.65	0.62
10^{-3}	0.65	0.62
10^{-4}	0.65	0.61
10^{-5}	0.64	0.62
10^{-6}	0.65	0.63
10^{-7}	0.65	0.62
10^{-8}	No growth	0.62

It is of course possible that the decreasing amounts of acid produced with decreasing amounts of culture is due to some unrecognized factor and not to a negative correlation between rapidity of growth and acid tolerance. If such is the case, it is still difficult to explain why the same phenomenon is not found in the serial 12-hour culture; and also why a smaller amount of acid is produced from a one cc. amount of this culture than from a similar amount of the parent culture.

One point should be mentioned, however, which made us somewhat skeptical about our interpretation of these facts—the marked decrease in amount of acid produced from an inoculum of 0.1 cc. as compared with 1 cc. of the original cultures. Though mathematically possible, it is difficult to believe that organisms which are present in numbers of less than ten per cc. in a 24-hour culture could increase sufficiently within seven days to have a measurable effect upon the final acidity. On the other hand, it is entirely possible that when the initial seeding of the slowly growing strains is very small they would be inhibited by the rapidly growing organisms, as was shown by Rogers[2] in a study of the inhibition of *Lactobacillus bulgaricus* by *Streptococcus lactis*.

In order to settle that point and test further our interpretation of these results another experiment was performed: Instead of using a 24-hour stock culture, it was tested after incubation for seven days at 37° C. If the higher acidity is produced by slower growing strains, there should be no

[2] Rogers, L. A. Jour. Bact. 16: 321. 1928.

difference in the amounts of acid produced from differing amounts of the culture under these conditions, as the slowly growing organisms should be present in large numbers. The results of this experiment with the two stock cultures are given in table 3.

TABLE 3

Acid Production by Varying Amounts of Aged Cultures of Streptococcus lactis

INOCULUM (CC.)	PER CENT ACID AS LACTIC ACID	
	Culture No. 8	Culture No. 21
1	0.79	0.77
10^{-1}	0.79	0.77
10^{-2}	0.79	0.77
10^{-3}	0.79	0.77
10^{-4}	0.79	0.77
10^{-5}	0.78	No growth
10^{-6}	0.79	No growth
10^{-7}	No growth	No growth

It will be noted from these data that in the case of a seven days' old culture, varying the amount of the inoculum did not change the final degree of acidity produced. This would appear to add distinct weight to the hypothesis that slow growth is associated with greater acid producing power.

A word of explanation should be added: This conclusion does not mean that there will be a rigid correlation between growth rate and acid production when various cultures of *Streptococcus lactis* are compared with each other. It is probable that many strains could be found which have slow growth rates and feeble acid producing power. However important the rate of growth may be, it is likely to prove of less significance than inheritance, even among bacteria.

SUMMARY

By segregating the fast growing strains from those which grow more slowly in stock cultures of *Streptococcus lactis*, it has been shown that slow growth is associated with greater acid tolerance.

The conclusion is drawn that such a relationship exists among strains of the same genetic constitution.

SOME PHYSICO-CHEMICAL PROPERTIES OF LACTOSE

I. THE SPONTANEOUS CRYSTALLIZATION OF SUPER-SATURATED SOLUTIONS OF LACTOSE

B. L. HERRINGTON

Department of Dairy Industry, Cornell University, Ithaca, N. Y.

During the past few years, research has been under way in the dairy chemistry laboratory at Cornell University directed toward the commercial production of beta lactose, an optical isomer of ordinary lactose. While working on that problem, the need for more data regarding the physical properties of lactose became evident. A search of the literature revealed that information on some subjects was entirely lacking; on others, the data were discordant; and in a few cases, the data found were suspected of error.

For these reasons, a series of researches were undertaken with the purpose of searching for errors in the literature, and of gathering new data which might serve as a basis for future research on lactose. The results of some of these investigations are reported in this series of papers on the physico-chemical properties of lactose.

No attempt will be made to give a complete review of the literature dealing with lactose. Such a review has already been published by Whittier (30). However, references to papers on special topics will be given as they are needed.

Before discussing the experimental work, it seems desirable to describe, briefly, the various modifications of lactose, and the terminology used to describe them. Lactose, or milk sugar, is a disaccharide having a free aldehyde group. Accordingly, two isomeric forms are known. The form having the higher optical rotation is known as alpha lactose; the form having the lower rotation is termed beta lactose. In aqueous solutions, an equilibrium is established between these two forms. The relative amounts of the two sugars present at equilibrium varies with the temperature. This mixture is referred to as equilibrium lactose, or as equilibrium mixture.

The lactose of commerce is a hydrate. In Hudson's early papers, this hydrate was regarded as having two hydroxyl groups on the terminal carbon atom. It was, therefore, related equally to both the alpha and beta forms. This view was opposed by Lowry (15) and later by Gillis (6), and it has apparently been abandoned by Hudson (10). In these papers, the hydrate of lactose is regarded as a true hydrate of the alpha form. It is referred to as alpha hydrate.

The anhydrous form of alpha lactose may be prepared by desiccation of the hydrate. It has never been prepared by direct crystallization. The

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beta form of lactose is the stable modification crystallizing from aqueous solutions at temperatures above 93° C. It forms anhydrous crystals; no hydrate is known.

INTRODUCTION

The control of the crystallization of lactose is of considerable industrial importance. It is necessary to prevent crystallization in ice cream, because it produces the defect known as sandiness (1, 35). It is necessary to prevent crystallization in spray milk powder in order to avoid caking (27). Moreover, the solubility of the powder is greatly decreased when the lactose crystallizes. A large part of the lactose is crystallized in sweetened condensed milk, and conditions are so regulated as to secure the smallest crystals possible. In the manufacture of lactose it is desirable to secure a maximum yield of crystals in a minimum of time, and to secure crystals which may be readily washed with a minimum of loss. The manufacture of each of these products presents crystallization problems of its own, and some of these problems have not yet been satisfactorily solved.

Because lactose is a mutarotating sugar, its solubility varies with time. This was recognized by Urech (28) in 1883, and was studied in detail by Hudson (8, 9). Moreover, lactose forms supersaturated solutions quite readily (3). These facts make a study of the crystallization of lactose more difficult, but more interesting. The results of such a study are reported in this section.

A study of supersaturated solutions must be based upon a knowledge of the true solubility of lactose. Gillis (7) has published a critical review of the values reported in the literature, together with some original data of his own. Sharp (22, page 149) has published a table of interpolated values from −30 to 100° C. at 10° intervals. His figures are based upon the data of Hudson and of Gillis.

The phenomena of crystallization have been studied by a number of workers. Unfortunately for our purpose, much of the work has been done on crystallization of melts of pure compounds. For the most part, the studies have dealt with one component systems but a lactose solution contains at least two components, if not three, and it is not known, for example, how many components should be considered in the crystallization of lactose in ice cream. Nevertheless much can be learned by reviewing the results obtained by those who worked with the more simple systems.

Supersaturated solutions have been an object of interest to many. Ostwald (21) reported that supersaturated solutions were of two kinds: those which required seeding before crystallization could take place, and those which could crystallize spontaneously. He termed the first class metastable, and the second class, representing a higher degree of supersaturation, he termed labile. Ostwald's nomenclature has been accepted by many but

there has been much dispute as to whether there is any real difference between the metastable and the labile states.

Jones and Shah (13) as a result of their study of solutions of KCl, KBr and KI believed in the existence of a definite metastable zone. Leighton and Peter (14), who worked with lactose, reported a "supersolubility curve" located 30° C. below the true solubility curve. However, Young and his coworkers (32, 33, 34) have presented considerable evidence to show that the boundary of the labile zone is not a definite one, but that its apparent position is dependent upon the degree of mechanical shock to which the solution is subjected. Jaffe (11) also reported the boundary of the labile zone an indefinite one. By repeated filtration and recrystallization, he was able to widen the metastable region. He attributed this to the removal of insoluble particles which acted as crystal nuclei.

The existence of metastable and labile zones may be explained in terms of the results of Tammann (26). He has shown that crystallization is dependent upon two independent factors; first, the appearance of crystal nuclei; second, the rate of growth of nuclei after they appear. He has also shown that as a melt is cooled below its freezing point, the probability of nuclei formation rises gradually to a maximum and then returns to zero. This last fact explains the apparent stability of glasses. They are so greatly supercooled that the probability of nuclei appearing is very small. According to Tammann, the velocity of crystal growth also passes through a maximum and approaches zero with sufficient supercooling. Now, if we say that the metastable zone is the region where spontaneous crystallization will not occur, we must consider it as the zone where the probability of nuclei formation is practically zero. On further cooling, the probability of nuclei formation increases and the solution passes into the labile zone where crystallization may be expected in a reasonable length of time. On still further supercooling, the chances of nuclei appearing decrease again, and we have solutions which may be considered as being in a glassy state. According to this view, there can be no sharp distinction between the labile and the metastable solutions. However, if the temperature coefficient of probability is sufficiently great, then there might appear to be a sharp line dividing the two zones.

It must be remembered that the degree of supersaturation is not the only factor governing the appearance of nuclei. The effect of mechanical shocks is often very great. Moreover, Richards (23) has demonstrated that something having the properties of a crystal nucleus may exist in solution for considerable periods of time. No evidence is available to show that this is true of lactose solutions, but it is worth investigating. It is interesting to note that Bothell (1) raised this question as far back as 1920 when he remarked that if one accepted the opinions which were current regarding the crystallization of lactose in ice cream, one would have to believe that

lactose solutions which had been crystallized once, were more readily crystallized a second time. There are a number of observations recorded in the literature which apparently support a theory of crystal nuclei resulting from the incomplete solution of crystals. As examples of this, Jones and Shah (13) found it necessary to boil solutions of alkali halides for ten minutes in order to destroy all crystal nuclei, and Miers and Isaac (20) found it necessary to heat solutions of sodium chlorate in boiling water for 48 hours in order to prevent premature crystallization. If it could be shown that lactose solutions must be heated, even after all lactose is in solution, to destroy these "ghosts" of crystals which induce crystallization on cooling, many anomalous results of crystallization might be explained.

The rate of growth of crystals is independent of the formation of nuclei. It is governed by the rate of diffusion to the crystal, and by the speed with which molecules can be oriented into the crystal lattice. (Marc (16, 17, 18, 19) has shown that the process of orientation can be distinguished experimentally from that of diffusion.) In the case of the crystallization of lactose, the rate of transformation of beta to alpha lactose may be a factor during the later stages (8). Sharp (22, page 153) has calculated the maximum velocity of crystallization at various temperatures as limited by this last factor. The influence of pH upon crystallization velocity has been measured experimentally by Jenkins (12) and by Troy and Sharp (27). Whittier and Gould (31) have published some experimental results showing the rate of crystallization when the solutions were agitated so vigorously that diffusion was a minor factor. But there is still need for information regarding the crystallization of lactose in the absence of agitation.

Marc (18, 19) showed that the presence of a third substance may influence the rate of crystallization from solution. Leighton and Peter (14) studied the influence of dyes upon the crystallization of lactose. Only three out of thirty-nine seemed to have any effect at all, and their action was slight. Fujimoto (5) reported that none of the certified dyes had any appreciable effect upon the crystallization of lactose, though sucrose and gelatine seemed to inhibit crystallization. On the other hand, Dahle (2) found no evidence that other sugars, or gelatine, had any influence on the crystallization of lactose in ice cream. He believed that the governing factor was the lactose content of the mix. This view seems widespread in spite of the fact that the concentration of lactose in frozen ice cream is independent of its concentration in the mix.

This study of supersaturated solutions was pursued with several objects in mind. It was desired to repeat the work of Leighton and Peter on supersolubility, without agitation, to determine whether there is a sharp line between the metastable and labile zones, and if it were found to locate it as definitely as possible. It was desired to determine at what degree of supercooling the probability of nuclei formation passed through a maxi-

mum, and to extend the investigation into the zone of glasses, if that were possible. It was hoped, also, that information might be gained regarding the rate of growth of individual crystals at various degrees of supercooling.

EXPERIMENTS

Leighton and Peter determined the degree of supercooling necessary before one crystal could initiate a general formation of crystals when the solution was shaken. In this study, efforts were made to determine the degree of supercooling necessary for nuclei to appear spontaneously, and to estimate the number which appeared. This made it necessary to prevent a general seeding of the solution by the first crystal to appear. Two methods were used to accomplish this: first, crystallization of lactose solutions in slender glass tubes; and second, crystallization in the presence of a gel which held the crystals in the place where they first appeared.

In the first experiments, lactose and water were weighed into a series of glass tubes, approximately two millimeters inside diameter, which were then sealed carefully. After heating in boiling water until all of the lactose was dissolved, the tubes were stored horizontally in a room kept at a constant temperature. When crystals appeared in these tubes, they fell to the side, but their path was so short that little opportunity was given for them to initiate a general crystallization. These tubes were examined at intervals with a hand lens, and when possible, the number of crystals or of clusters was determined.

This method had the disadvantage that only very small volumes of solutions could be used, approximately 0.3 grams in each tube. For this reason, it was feared that crystallization might not occur in some of the experiments where it would take place in a larger volume of solution under the same conditions. However, several experiments were carried out by this method. The results of one experiment, where the crystallization was allowed to take place at 30° C., are shown in table 1. The results of these experiments are in agreement with those of Leighton and Peter who found that solutions must be supercooled 30° C. in order to initiate crystallization. However, it might be stated that in another experiment, tubes 15, 12 and 11 did not crystallize after 68 days at 0° C. where the supercooling was as great as 53° C. Tube 8 contained only one cluster at that time. These results indicate that the supersolubility curve is at a higher degree of supercooling in the case of the more dilute solutions.

In order to work with larger volumes of solution, another method was devised. Preliminary experiments indicate that 0.7 per cent of agar agar had no appreciable effect upon the crystallization of lactose, and it gave a gel firm enough to hold crystals in suspension. (Friedman (4) found that the diffusion of lactose was retarded only slightly in such a gel.) Accordingly, a 0.7 per cent solution of agar agar was prepared and carefully

filtered. The lactose was dissolved in this solution, and from the weights of the two components, the temperature of saturation was estimated using the table of solubilities compiled by Sharp (22, page 149). Ten cc. portions of these solutions were placed in test tubes and covered with vaseline to prevent evaporation. The test-tubes were heated for three minutes in boiling water to destroy all crystal nuclei, and then placed in thermostats maintained at suitable temperatures. In the case of the tubes held at 40° C. and at 50° C., it was necessary to cool the tubes momentarily to 37° C., to allow the agar agar to set. In order to reduce experimental errors, five tubes were used for each solution at each temperature.

Figure 1 shows the solubility curve of lactose, and the mean time ex-

TABLE 1

The appearance of crystal nuclei at 30° C., in solutions saturated at various temperatures

TUBE NUMBER	SATURATION TEMPERATURE OF	NUMBER OF CRYSTALS PRESENT				
		10 hours	34 hours	58 hours	136 hours	360 hours
2	96° C.	TMC	TMC	TMC	TMC	TMC
7	90	TMC	TMC	TMC	TMC	TMC
10	86	TMC	TMC	TMC	TMC	TMC
3	84	29–20	TMC	TMC	TMC	TMC
4	80	38– 3	TMC	TMC	TMC	TMC
1	75	22– 1	TMC	TMC	TMC	TMC
14	66	1	2	30– 2	TMC	TMC
13	61	1	1	0	0	TMC
9	54	0	0	0	0	0
15	53	0	0	0	1	0
12	51	0	0	0	0	0
8	46	0	1	1	0	0
11	46	0	0	1	0	0

TMC = too many to count.

Double numbers = single crystals first, clusters second.

pressed in hours for crystallization to commence at various degrees of supercooling. In one or two cases, one tube out of a set failed to crystallize even after the lapse of three thousand hours. Such tubes were disregarded in calculating the mean time for nuclei to appear. In the great majority of cases, the agreement of duplicates was quite satisfactory. The cases where no crystals appeared in any one of the set of five tubes after 3000 hours are designated by an interrogation mark. The tubes were examined for the presence of crystals by revolving before a bright light. The reflection of light from the crystals faces made possible the detection of even minute crystals.

A study of these data indicates that the higher the initial concentration of the solution, the less supercooling is possible before nuclei formation

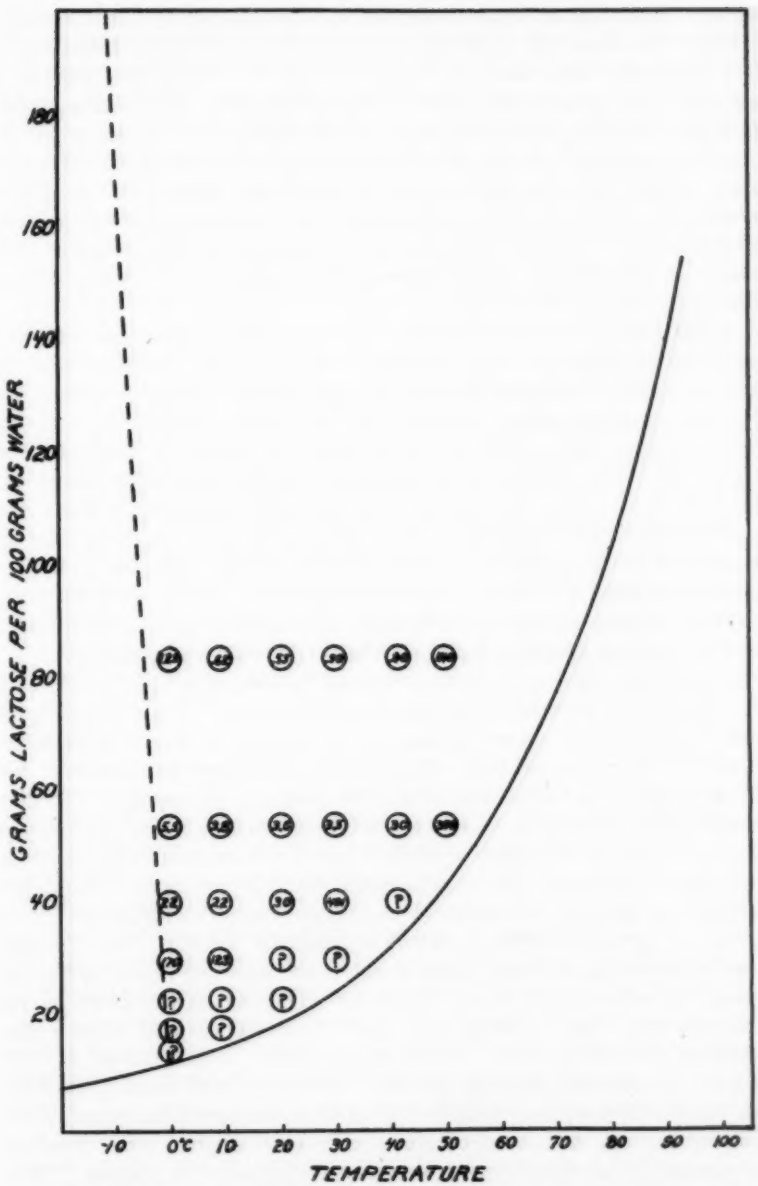

FIG. 1. THE SOLUBILITY CURVE OF LACTOSE, AND THE HOURS REQUIRED FOR CRYSTALS TO APPEAR AT VARIOUS DEGREES OF SUPERCOOLING.

? indicates no crystals after 3000 hours.

begins. This is in agreement with the results obtained by the other procedure. The data also show that the probability of nuclei formation is very small with slight degrees of supercooling, but it rises to a maximum and then falls again as the supercooling is increased. This fall is shown quite plainly in the case of the solutions saturated at 77° C. and at 57° C. It was not possible to cool the other solutions sufficiently to demonstrate this effect. These data also substantiate the belief that there is no real difference between the labile and the metastable zone with respect to spontaneous crystallization. There is no region of supersaturation which can be considered as permanently stable, although the probability of crystallization taking place may be very small.

Table 1 shows that the tendency to form clusters increases with the degree of supercooling. Examination of the test tubes indicated that, in general, there is a greater tendency to form clusters at low temperatures, with a given degree of supercooling, and that clustering increases with the degree of supercooling. This may explain the results of Leighton and Peter (14). They may have measured the cluster forming tendency instead of the tendency toward spontaneous nuclei formation. When the supercooling exceeded a certain value, new crystals would begin to form on the surface of the parent crystal and these would be broken away by the agitation to form new nuclei for crystal growth. If this is the true explanation of their results, then their data are applicable to those cases where there is vigorous agitation during crystallization but not elsewhere.

It is of some interest to extend the results shown in figure 1, to the case of lactose solutions held at temperatures below zero. Even though pure aqueous solutions of lactose can scarcely be expected to behave in the same manner as ice cream, yet some valuable suggestions may be obtained. We will assume that the water crystallizes out promptly on cooling. The first point to be considered is that after ice begins to form, the concentration of the solution is independent of its original concentration and depends only upon the temperature. Furthermore, after the ice begins to form, the unfrozen syrup may be considered as though it were a solution saturated at a much higher temperature than was originally the case. It is possible to estimate the approximate composition of the unfrozen lactose syrups at various temperatures by means of the known molecular depression of the freezing-point. The dotted line of figure 1, shows the approximate concentration of such syrups at various temperatures. It is apparent, at once, that the experimental data fall in a quite different part of the diagram from that which represents the conditions in an ice cream hardening room. However, we may say that at temperatures only a few degrees below freezing, the chances of spontaneous crystallization do not seem very great. With further supercooling, the probability of crystallization increases and must pass through a maximum although it is not possible to determine the posi-

tion of that maximum from the data now available. At still lower temperatures, the solution would become more stable since it is moving to the left on the chart while the point of maximum nuclei formation seems to be moving toward the right as the concentration increases.

One other point of interest might be considered in connection with figure 1. Let us suppose that a solution in equilibrium at $-30°$ C. is warmed to $-20°$ C. This might be done in either of two ways. If the temperature change occurs very slowly, ice would melt as the temperature rises, diluting the solution. The point on the diagram representing the solution would move downward along the dotted line to the new temperature. On the other hand, if the solution were warmed more rapidly than the melting ice could diffuse into the syrup, then the point representing the system would not move down along the line, but would move directly out to the $-20°$ C. abscissa. It is not yet possible to predict the relative probabilities of nuclei formation at these two points, but if it should happen to be much greater in the second case than in the first, then the use of the term "heat shock" would be justified, and the need for preventing rapid fluctuations in storage temperature would be explained.

In the case of ice cream, the effect of the sucrose must be considered. In frozen ice cream, the total concentration of sugars is independent of the original sugar concentration and depends only upon the temperature. The concentration of lactose, however, would depend upon the ratio of lactose to sucrose as well as upon the temperature. In an average ice cream mix, the concentration of lactose in water would be only about one-fourth as great as the concentration in a pure lactose solution at the same temperature.

In a frozen mix, the concentration of lactose depends upon the lactose sucrose ratio. This fact might well be emphasized for it is one of the most important factors governing sandiness in ice cream. Furthermore this effect of sucrose has been overlooked entirely by those who have investigated the effect of sucrose upon the crystallization of lactose above the freezing point.

These experiments on crystallization also gave information regarding the rate of crystal growth at various degrees of supercooling in the absence of agitation. Accurate measurements were not possible, without disturbing the original experiment, but observations showed that if the solutions were cooled too much, the rate of crystal growth was greatly diminished, figure 2. The maximum rate of crystallization was at approximately 30° C. This agrees, in general, with the calculations of Sharp (22, page 153), and with the experiments of Whittier and Gould (31). In their cases, the limiting factor was the rate of transfer of beta to alpha lactose. However, in these experiments, the crystallization was so slow that mutarotation velocities must have been of secondary importance. The slow growth at the lower

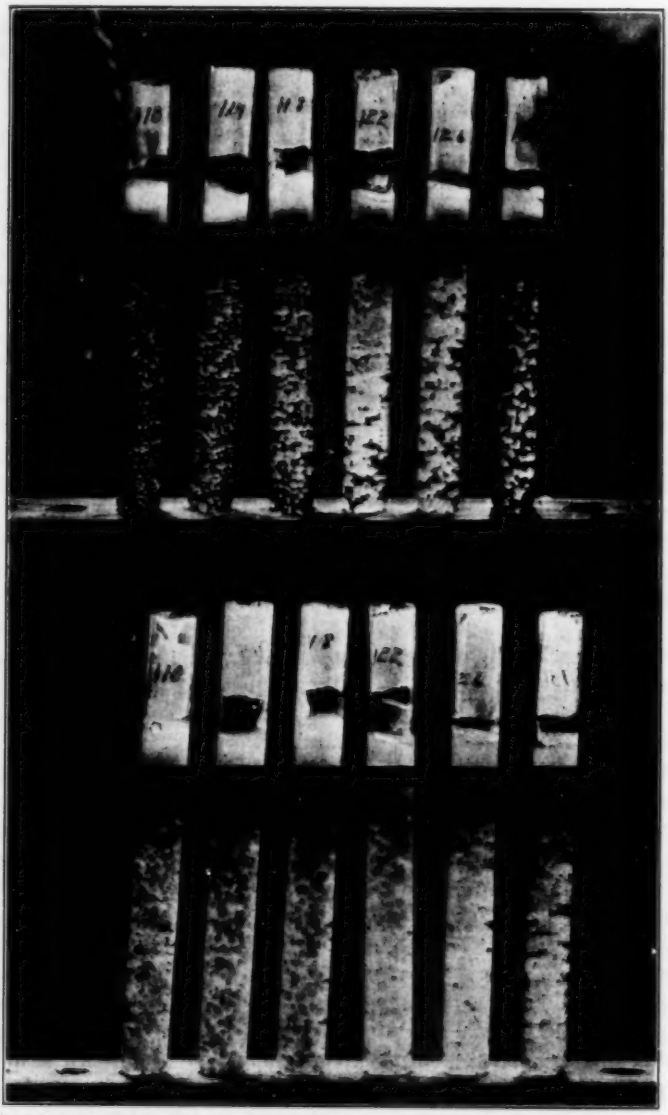

FIG. 2. THE EFFECT OF TEMPERATURE UPON THE RATE OF CRYSTAL GROWTH.

Two photographs of six tubes containing a lactose solution saturated at 77° C., each tube having been stored at a different temperature. From left to right, tubes were stored at 0°, 10°, 20°, 30°, 40°, and 50° C. The photographs were taken after 16.5 and 70 hours.

temperatures was probably due to a lower diffusion velocity of lactose in solution, and to a lower speed of orientation of molecules into the crystal lattice. Both of these factors would increase enormously in importance when a part of the water was frozen and therefore it seems reasonable to assume that in ice cream, the slow speed of mutarotation is of little importance. Mutarotation does place an upper limit to the rate of crystallization of lactose, but that maximum rate is probably never approached in ice cream. In any case, even without mutarotation, it would be possible to have two or three per cent of sand in ice cream which would be sufficient to render it unmarketable.

In spray powders, the lactose is not usually crystallized, yet the water content is so low that the solution is practically a solid (27). The same must be true of ice cream at storage temperatures. It is not possible to prepare such concentrated solutions by dissolving lactose directly in water, therefore other methods were devised by which they could be prepared in the laboratory for study.

According to theory, these glasses might be prepared by cooling a concentrated lactose solution rapidly enough to pass through the metastable and labile zones before crystallization began. The glasses could then be concentrated further, if desired, by desiccation at low temperatures. In practice, about eight grams of lactose was placed in a 250 cc. Erlenmeyer flask with about 15 cc. of water. The solution was then boiled down over a free flame until there was imminent danger of crystallization. The flask was then connected to a vacuum oven containing a tray of calcium chloride. When the stop-cock was opened, the lactose solution was converted into a foam which became solid in a few seconds because of the rapid cooling and evaporation. The vacuum pump (Hyvac) was allowed to run about one-half hour before the flask was disconnected. Then a glass marble was placed in the flask and it was tightly stoppered. The flasks used were of very heavy pyrex glass. With careful shaking, it was possible to reduce the glassy lactose to a fine powder which could easily be transferred to another container for storage.

It might be mentioned that success in the preparation of lactose glasses by this method seemed to depend partly upon the flask used. In some flasks, crystallization invariably began too soon, while boiling off the excess water. There was no visible difference in the nature of the glass surfaces which could account for this.

Under the microscope, these lactose preparations resembled ground glass. When examined by polarized light, some of the preparations seemed entirely free from crystalline material. Other batches showed a small amount of doubly refracting material. These glasses absorb moisture rapidly from the air, and under the microscope, they appear to melt down to a liquid and then recrystallize into solid cakes—see figure 3. Schmoeger (24) observed

this behavior of lactose more than 50 years ago. More recently, Troy and Sharp (27) have suggested that this is the explanation of the caking of milk powders and of the peculiar hydration-dehydration curves found by Supplee (25) in his study of milk powders.

For some purposes, glasses were also prepared by drying lactose solutions rapidly on a Mojonnier machine in accordance with the procedure used for solids in milk. This method had the disadvantage that only small amounts, 0.2 gram, could be prepared at one time without having crystallization occur, and it was practically impossible to recover the glass from the dish.

The moisture content of the glasses, as prepared by the first method, ranged from about 3 per cent to about 8 per cent. This was determined in two ways. First, by weighing the flask plus lactose, and later weighing the flask plus glass; and second, by drying the glass in a vacuum over P_2O_5. These moisture contents correspond to lactose concentrations as high as 3200 grams of anhydrous sugar per 100 cc. of water. High as this figure seems to be, it corresponds very well to conditions as they exist in a spray powder.

Lactose glasses give up most of their water more rapidly than does alpha hydrate. This was clearly shown by one experiment in which samples of powdered lactose glass, and of alpha hydrate, having approximately the same particle size were dried in a vacuum at room temperature over P_2O_5. Figure 4, shows the rates of loss in weight.

The increased rate of loss by the glass probably accounts for the speed with which a Mojonnier solid's residue can be dried to an apparently constant weight. However, since lactose glass is a solution, constancy of weight does not mean complete dryness but merely the establishment of an equilibrium. The lactose glass used in this experiment appeared to contain only 6.0 per cent of moisture but it continued to lose weight, very slowly, until a total loss of 6.65 per cent was reached. At this point, the vacuum oven was heated to 100° C. and held at that temperature for two weeks. After this treatment, the total loss was 7.09 per cent. The glass showed no discoloration in spite of the treatment which it had received. On the other hand, the crystalline lactose had lost only 5.09 per cent of its original weight but it had become brown.

Lactose glasses of low moisture content may be kept in a desiccator for indefinite periods without crystallization. If exposed to the air, they take up water until crystallization begins, whereupon they lose their excess moisture. Usually alpha hydrate is formed, but in some cases, the product was apparently beta lactose. Figure 5 shows some typical hydration-dehydration curves of pure lactose glasses. The glasses were prepared by dissolving a known weight of lactose in two cc. of water and then drying the solution rapidly by the Mojonnier procedure (evaporation of free water

FIG. 3. SHOWING THE ABSORPTION OF MOISTURE AND THE SUBSEQUENT CRYSTALLIZATION OF A SAMPLE OF LACTOSE GLASS.

A—glass; B—solution; C—crystallized sugar.

on a hot plate at 185° C., followed by drying in a vacuum oven at 100° C.). The dish containing the residue was weighed on a chainomatic balance and then a beaker containing water was placed in the balance case. The door of the balance was left raised about one centimeter. Weighings were made every ten minutes until the changes in weight became so small that the periods were extended. In some cases, the final weights approached 100 per cent of the original weight, indicating the formation of alpha hydrate. In other cases, the final weights were much lower indicating the formation of beta lactose. In different experiments percentages of 96.02, 96.56 and 95.15 were obtained. In each of these cases, the sample was still losing weight when the experiment was discontinued. It seemed probable that they were approaching the limiting value of 95 per cent corresponding to the formation of beta lactose. Direct evidence for the presence of beta lactose is lacking, but there is no reason why beta lactose might not be formed under these conditions, particularly if the glass became seeded with beta during the initial heating period.

It was hoped that the hydration curves would indicate how much moisture was necessary before crystallization could take place at room temperature. However, no agreement was found between the maximum moisture contents reached in the different experiments. This was not surprising, since the method used measured the mean moisture content of the sample,

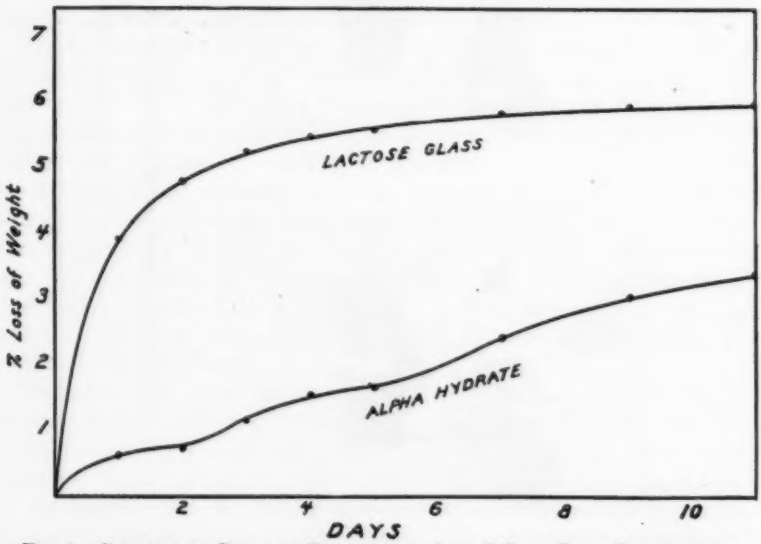

FIG. 4. COMPARATIVE RATES OF DEHYDRATION OVER P_2O_5 AT ROOM TEMPERATURE, UNDER VACUUM.

Actual moisture contents: glass, 7.09%; hydrate, 5.09%.

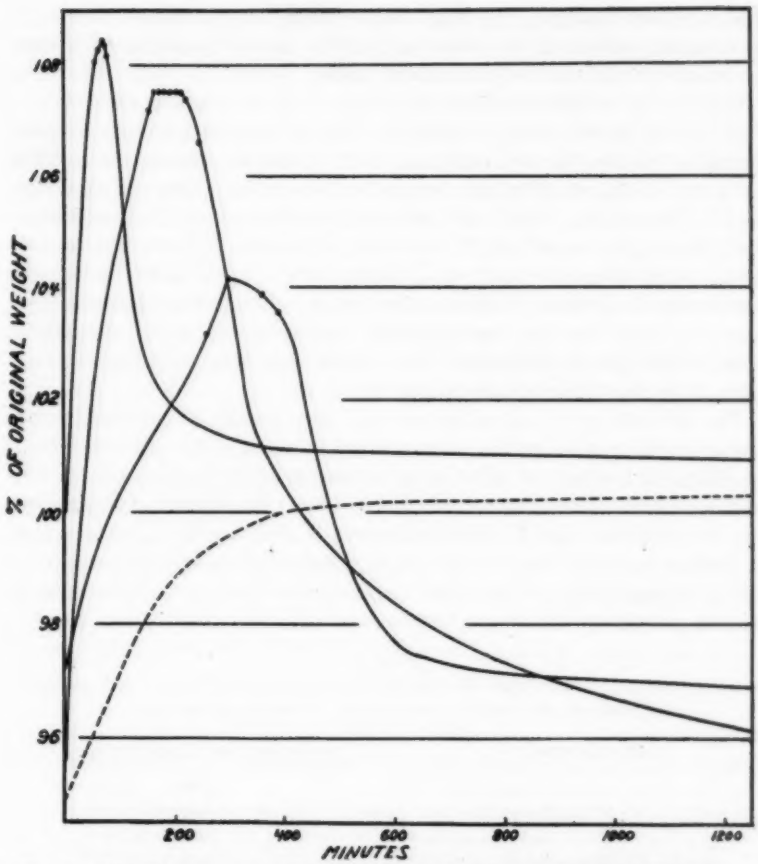

FIG. 5. THE HYDRATION AND DEHYDRATION OF LACTOSE AT CONSTANT HUMIDITY. Solid lines represent glasses; dotted lines, alpha anhydride.

while crystallization was determined by the moisture content of the most dilute part of the glass. For that reason, high values of moisture content at the beginning of crystallization are more significant than low values. It might be possible to obtain concordant results by allowing the glass to take up moisture very slowly, but it was not considered that such a method would prove very satisfactory and it was not tried.

The crystallization of lactose may be brought about by the addition of alcohol to aqueous solutions of the sugar. This method was frequently employed, in the preparation of pure lactose, before the complex nature of lactose solutions was understood. As an example of this, Walker (29), in his study of the determination of reducing sugars, prepared a "precipi-

tated'' lactose $C_{12}H_{22}O_{11} \cdot \frac{1}{2} H_2O$. Accordingly, he published tables for the reducing power of hydrated, anhydrous and of precipitated lactose. His product contained 2.43 per cent of water.

Casual observation indicated that ''precipitated'' lactose must vary a great deal in its composition, which is a fact of some importance in interpreting the results of some of the early work on lactose. In order to confirm this, a microscopic examination was made of the crystals obtained by adding different volumes of 95 per cent ethanol to saturated solutions of lactose. When the proportion of alcohol was low, only alpha hydrate was precipitated. When larger proportions of alcohol were used, beta lactose was also thrown out of solution. Unless a very large volume of alcohol was used, it seems certain that the ''precipitated'' lactose would always contain an excess of the alpha modification. This seems to be true of Walker's preparation if we may judge by the moisture content.

The fact that precipitated lactose may vary greatly in composition was shown clearly by an experiment in which 5 volumes of 95 per cent ethanol was added to a saturated solution of lactose at approximately 0° C. The resulting solution was analyzed for total solids at 15 minute intervals. The data are given in table 2. The concentration fell rapidly at first, due to the precipitation of alpha lactose. Then, after an interval of thirty minutes without change, beta lactose began to precipitate though the separation of beta was not as rapid as that of alpha.

TABLE 2

The precipitation of lactose after the addition of five volumes of 95 per cent ethanol to one volume of saturated aqueous solution at a temperature of 0° C.

TIME IN MINUTES	PER CENT OF SOLIDS IN SOLUTION
0	2.08
15	1.13
30	1.10
45	1.10
60	0.877
75	0.526
90	0.388
105	0.289
120	0.249
180	0.210

SUMMARY

Lactose solutions may be supercooled greatly without crystallization taking place. The degree of supercooling necessary for crystallization to occur, in the absence of agitation, is less for concentrated solutions than for dilute solutions.

There is no sharp line dividing the metastable and labile zones in the case of supersaturated lactose solutions.

As a solution of lactose is supercooled, the rate of nuclei formation passes through a maximum. The temperature of most rapid nuclei formation is higher in the case of concentrated solutions.

The rate of crystal growth passes through a maximum as the temperature is lowered. At low temperatures, the rate of crystallization, in the absence of agitation, is so slow that mutarotation cannot be the limiting factor.

Lactose solutions may be prepared which are so greatly supersaturated as to resemble solids. Such glasses are stable at room temperature, if protected from moisture.

Lactose glasses are hygroscopic, absorbing moisture from the air until sufficiently dilute for crystallization to take place.

Lactose glasses are supersaturated with respect to both alpha hydrate and beta anhydride. Either modification may appear when such glasses crystallize.

When desiccated, lactose glasses lose a part of their moisture quickly, coming to an apparently constant weight. This probably accounts for the success of the Mojonnier method of determining total solids in milk. The removal of the last portions of water from such glasses is very slow.

Lactose precipitated by alcohol is not equilibrium lactose, though it may resemble it closely. Alpha lactose is more readily precipitated from solution by alcohol than is beta lactose. Consequently, precipitated lactose varies in composition. It usually contains a greater proportion of alpha lactose than is present in the equilibrium mixture.

REFERENCES

(1) Bothell, F. H. Facts about sandy ice cream. Ice Cream Trade Journal 16 (2): 55–56. 1920.

(2) Dahle, Chester D. Sandy ice cream. Proceedings World's Dairy Congress 1: 500–10. 1924.

(3) Dubrunfaut, ———. Note sur le sucre de lait. Compt. rend. 42: 228–33. 1856.

(4) Friedman, Leo. Structure of agar gels from studies of diffusion. J. Am. Chem. Soc. 52: 1311–4. 1930.

(5) Fujimoto, A. T. Some factors influencing the rate of crystallization of lactose in ice cream. M.S. Thesis. Ohio State University. 1926.

(6) Gillis, J. Nouvelles recherches sur le sucre de lait. Rec. trav. chim. 39: 88–125. 1920.

(7) Gillis, J. Solubilité du sucre de lait. Rec. trav. chim. 39: 677–8. 1920.

(8) Hudson, C. S. The hydration of milk sugar in solution. J. Am. Chem. Soc. 26: 1065–82. 1904.

(9) Hudson, C. S. Further studies on milk sugar. J. Am. Chem. Soc. 30: 1767–83. 1908.

(10) Hudson, C. S., and Hockett, R. C. A novel modification of lactose. J. Am. Chem. Soc. 53: 4455–56. 1931.

(11) Jaffé, George. Studien an übersattigen Lösungen. Z. physik. chem. 43: 565–94. 1903.

(12) JENKINS, J. D. The effect of various factors upon the velocity of crystallization of substances from solution. J. Am. Chem. Soc. 47: 903–22. 1925.

(13) JONES, B. M., AND SHAH, P. G. The spontaneous crystallization of solutions of potassium chloride, potassium bromide, and potassium iodide. J. Chem. Soc. 103: 1043–52. 1913.

(14) LEIGHTON, A., AND PETER, P. N. Factors influencing the crystallization of lactose. Proceedings World's Dairy Congress 1: 477–85. 1924.

(15) LOWRY, T. M. Studies of dynamic isomerium. III. Solubility as a means of determining the proportions of dynamic isomerides in solution. Equilibrium in solutions of glucose and galactose. J. Chem. Soc. 85: 1551–70. 1904.

(16) MARC, ROBERT. Über die Krystallization aus wässerigen Lösungen. Z. physik. Chem. 61: 385–98. 1908.

(17) MARC, ROBERT. Über die Krystallization aus wässerigen Lösungen. II. Z. physik. Chem. 67: 470–500. 1909.

(18) MARC, ROBERT. Über die Krystallization aus wässerigen Lösungen. III. Z. physik. Chem. 68: 104–14. 1909.

(19) MARC, ROBERT. Über die Krystallization aus wässerigen Lösungen. IV. Z. physik. Chem. 73: 685–723. 1910.

(20) MIERS, H. A., AND ISAAC, FLORENCE. The refractive indices of crystallizing solutions with special reference to the passage from the metastable to the labile condition. J. Chem. Soc. 89: 413–54. 1906.

(21) OSTWALD, W. Studien über die Bildung und Umwandlung fester Körper. I. Übersattigung und Überkaltung. Z. physik. Chem. 22: 289–330. 1897.

(22) RAHN, OTTO, AND SHARP, PAUL FRANCIS. Physik der Milchwirtschaft. Paul Parey, Berlin. 1928.

(23) RICHARD, WILLIAM T. Persistence and development of crystal nuclei above the melting point. J. Am. Chem. Soc. 54: 479–95. 1932.

(24) SCHMÖGER, M. Ueber wasserfreien Milchzucker. Ber., 14: 2121–6. 1881.

(25) SUPPLEE, G. C. Humidity equilibria of milk powders. JOUR. DAIRY SCI. 9: 50–61. 1926.

(26) TAMMANN, G. Über die Abhängigkeit der Zahle der Kerne, welche sich in verschiedenen unterkühlten Flussigkeiten bilden, von der Temperatur. Z. physik. Chem. 25: 441–79. 1898.

(27) TROY, H. C., AND SHARP, PAUL FRANCIS. Alpha and beta lactose in some milk products. JOUR. DAIRY SCI. 13: 140–57. 1930.

(28) URECH, F. Ursachlicher zusammenhang zwischen Loslichkeits und optischer Drehungserscheinungen bei Milchzucker und Formulirung der Uebergangsgeschwindigkeit seiner Birotation in die Normale. Ber., 16: 2270–71. 1883.

(29) WALKER, PERCY H. The unification of reducing sugar methods. J. Am. Chem. Soc. 29: 541–554. 1907.

(30) WHITTIER, E. O. Lactose, a review. Chem. Reviews 2: 85–125. 1925.

(31) WHITTIER, E. O., AND GOULD, S. P. Speed of crystallization of lactose, galactose, glucose, and sucrose from pure solutions. Ind. Eng. Chem. 23: 670–3. 1931.

(32) YOUNG, S. W. Mechanical stimulus to crystallization in supercooled liquids. I. J. Am. Chem. Soc. 33: 148–62. 1911.

(33) YOUNG, S. W., AND CROSS, R. J. Mechanical stimulus to crystallization. II. J. Am. Chem. Soc. 33: 1375–88. 1911.

(34) YOUNG, S. W., AND VAN SICKLEN, W. J. The mechanical stimulus to crystallization. J. Am. Chem. Soc. 35: 1067–78. 1913.

(35) ZOLLER, H. F., AND WILLIAMS, O. E. Sandy crystals in ice cream: their separation and identification. J. Ag. Research 21: 791–5. 1921.

INFLUENCE OF HOMOGENIZATION ON THE CURD TENSION OF MILK*

D. R. Theophilus, H. C. Hansen, and M. B. Spencer
Department of Dairy Husbandry, University of Idaho, Moscow, Idaho

Since the work of Hill (1), 1923, considerable attention has been given to the study of soft curd milk. Although it is possible to modify the curd character of milk by heating, natural soft curd milk is at present practically the sole source of supply.

As early as 1916 Washburn and Jones (8) studied the values of different grades of milk for infant feeding using pigs as experimental animals. After studying the effect of homogenization they concluded, "The homogenization of the fat does not seem to be helpful, but the casein of the milk is sufficiently modified by the application of this process so that possible benefit may result from its employment." Ladd (4), 1915, Washburn (9), 1931, Wallace (7), 1932, and Weisberg *et al.* (10), 1933, report that homogenization lowers the curd tension of milk. In fact, the National Dairy Products Corporation, Inc., was granted a patent (6) in January, 1932, covering the production of soft curd milk by homogenization. Dr. Shrader (5), president of the Research Laboratories of the company, states that the patent office will probably cancel this patent as the company has declined to reply to a citation.

Just what relationship exists between the pressure of homogenization and the curd tension of milk has never been established. This work was undertaken in an effort to determine this relationship.

PROCEDURE

Milk used in this work was from cows in the University of Idaho dairy herd, composed of Holsteins and Jerseys. In no instance was any milk used from cows having any physical or bacteriological symptoms of mastitis.

Part I

Thirty gallons of milk were divided into six split batches. Each of the five-gallon batches was heated to 130° Fahrenheit and then homogenized at a selected pressure in either a single-stage or two-stage homogenizer.

Homogenization pressures of 500, 1,000, and 2,000 pounds were used. The two-stage homogenizer was operated with the second valve at three-fourths the pressure of the first valve.

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Curd tension was determined within one-half hour after homogenization by the method recommended by Hill (2). Ten trials were run.

Part II

Five gallons of milk were selected from each of three groups of three cows, one group producing relatively high, one medium, and one low curd tension milk. Milk with a curd tension above 60 grams was considered as relatively high curd tension milk; 30 to 60 grams, relatively medium curd tension; and below 30 grams, low or soft curd milk. Each lot of milk from the three groups of cows was heated to 130° Fahrenheit and homogenized at 1,000 pounds' pressure in a single-stage homogenizer.

Curd tension was determined as in Part I. Ten trials were run with each group.

PRESENTATION OF DATA

Part I

Effect of type of homgenizer and pressure of homogenization

The curd tension of milk was reduced by homogenization, using either a single-stage or a two-stage homogenizer at pressures of 500, 1,000, and 2,000 pounds (Table 1, Figure 1). Increases in homogenizing pressures progressively reduced the curd tension.

A pressure of 500 pounds on the single-stage and two-stage homogenizers reduced the curd tension 28.4 and 20.8 per cent respectively. The effect,

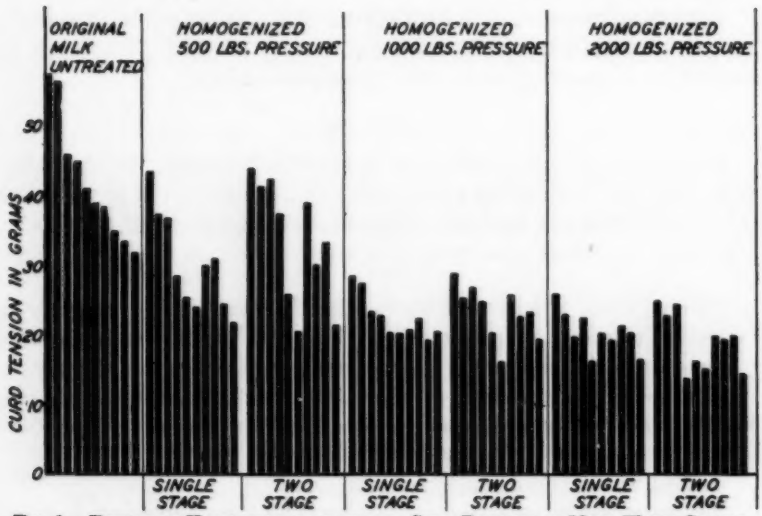

FIG. 1. EFFECT OF HOMOGENIZATION ON THE CURD TENSION OF MILK WHEN SINGLE-STAGE AND TWO-STAGE HOMOGENIZERS WERE USED AT DIFFERENT PRESSURES.

TABLE I

Effect on curd tension of single-stage and two-stage homogenizers at different pressures

TRIAL NUMBER	ORIGINAL MILK	500 LBS. PRESSURE		1,000 LBS. PRESSURE		2,000 LBS. PRESSURE	
		Single-Stage	Two-Stage	Single-Stage	Two-Stage	Single-Stage	Two-Stage
Average (mean)	42.4 ± 2.8	30.4 ± 2.1	33.6 ± 2.9	22.8 ± 1.0	23.6 ± 1.2	20.7 ± 0.9	19.3 ± 1.3
Standard Deviation ...	8.5 ± 1.9	6.4 ± 1.4	8.8 ± 1.9	3.1 ± 0.7	3.6 ± 0.8	2.7 ± 0.6	3.8 ± 0.8
Per cent Decrease		28.4	20.8	46.4	44.3	51.3	54.6

TABLE II

Effect of homogenization on relatively high, medium, and low curd tension milk

(Single-Stage Homogenizer, 1,000 lbs. Pressure)

TRIAL NUMBER	HIGH CURD TENSION GROUP		MEDIUM CURD TENSION GROUP		LOW CURD TENSION GROUP	
	Original	Homogenized	Original	Homogenized	Original	Homogenized
Average (mean)	67.9 ± 2.7	35.7 ± 2.6	46.2 ± 1.9	28.5 ± 1.4	38.6 ± 1.8	27.4 ± 1.4
Standard Deviation	8.3 ± 1.9	7.9 ± 1.8	5.6 ± 1.2	4.3 ± 0.9	5.4 ± 1.2	4.3 ± 0.9
Per cent Decrease	48.5		38.3		28.9	

however, was particularly noticeable when 1,000 pounds' pressure was used for the curd tension was decreased 46.4 per cent with the single-stage homogenizer and 44.3 per cent with the two-stage homogenizer. A further reduction in curd tension, approximately 5 and 10 per cent, or 2 and 4 grams respectively, resulted when the pressure on both homogenizers was increased from 1,000 to 2,000 pounds. The reduction in curd tension was not proportional to the increase in homogenization pressure.

There was apparently little difference between the single-stage and the two-stage homogenizers in their ability to reduce the curd tension. The single-stage homogenizers gave a slightly greater reduction in curd tension at both 500 and 1,000 pounds' pressure than did the two-stage homogenizer. At 2,000 pounds' pressure, however, the two-stage homogenizer caused a slightly greater reduction in curd tension.

These results demonstrate that: first, the curd tension of milk is reduced by homogenization at 500, 1,000, and 2,000 pounds' pressure; second, the percentage reduction in curd tension increases with an increase in homogenizing pressure, but the increase in percentage reduction of curd tension is not proportional to the increase in pressure of homogenization; and third, there is no appreciable difference in the efficiency of single and two-stage homogenizers in the reduction of the curd tension of milk at pressures used in this work.

Part II

Effect of homogenization on high, medium, and low curd tension milk

The results of homogenizing relatively high, medium, and low curd tension milk in a single-stage homogenizer at 1,000 pounds' pressure are shown in table 2, figure 2. A single-stage homogenizer was used in this phase of the work as it was the only one available at the time. The results, however, are comparable to those which may be expected from a two-stage homogenizer. A pressure of 1,000 pounds was used because it had been suggested by commercial workers and because there was little difference between the results obtained with 1,000 and 2,000 pounds' pressure.

Results indicate that the percentage reduction in curd tension increases with an increase in the curd tension of the unhomogenized milk. The average percentage reduction in 10 trials on relatively low, medium, and high curd tension milk was 28.9, 38.3, and 48.5 per cent respectively.

DISCUSSION OF RESULTS

Considerable interest has developed in the use of soft curd milk for infant feeding. If soft curd milk continues to merit the attention of milk distributors, more extensive developments along this line could be expected if curd tension could be regulated by processing the milk rather than selecting milk from cows naturally producing soft curd milk.

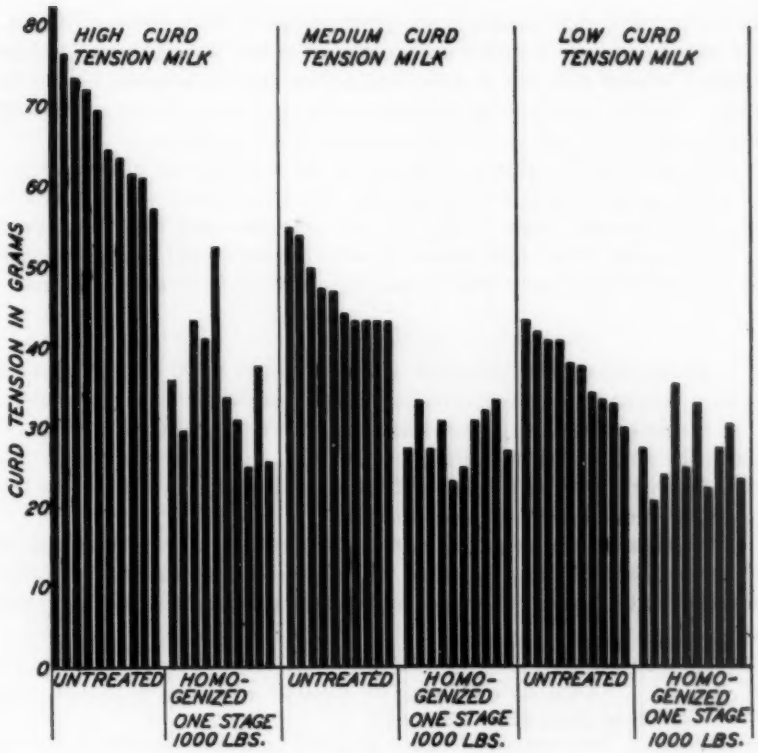

FIG. 2. COMPARISON OF HIGH, MEDIUM, AND LOW CURD TENSION MILK BEFORE AND AFTER HOMOGENIZATION AT 1000 POUNDS PRESSURE, WITH A SINGLE-STAGE HOMOGENIZER.

Data presented show that the curd tension of milk can be decreased by homogenization. There is apparently little difference between the single-stage and the two-stage homogenizers in their effectiveness in lowering the curd tension of milk. Increasing the pressure of homogenization decreases the curd tension, but the decrease in curd tension is not proportional to the increase in pressure of homogenization. If 500 pounds' pressure is used, a reduction of about 25 per cent in curd tension may be expected; with 1,000 pounds, a reduction of about 46 per cent; and with 2,000 pounds, about 53 per cent. Results obtained with pressures of 1,000 and 2,000 pounds agree with the work of Wallace (4).

Results also indicate that the higher the curd tension of the unhomogenized milk, the greater will be the percentage reduction in curd tension.

The reduction in curd tension is not great enough to make low curd tension milk out of milk with a curd tension above 60 grams, but will usually make low curd tension milk out of milk with a curd tension between 30 and 60 grams. Medium curd tension milk was reduced in curd tension sufficiently to fall in the low or soft curd class in 23 out of 31 instances.

Although Hill (3) questions the use of the homogenizer in the production of soft curd milk, this work indicates that homogenization has commercial possibilities and might be used for two purposes: first, to further lower the curd tension of low curd tension or soft curd milk; and second, to make soft curd milk out of milk with a curd tension between 30 and 60 grams.

SUMMARY

1. Homogenization at pressures of 500, 1,000, or 2,000 pounds reduced the curd tension of milk approximately 25, 46, and 53 per cent respectively.

2. Single-stage and two-stage homogenizers were equally effective in reducing the curd tension of milk.

3. The higher the curd tension of the original milk, the greater was the percentage reduction after homogenization.

4. Homogenization might be used to further lower the curd tension of soft curd milk or to make soft curd milk out of milk of medium curd tension (30 and 60 grams), or reduce high curd tension milk to medium curd tension.

REFERENCES

(1) HILL, R. L. A test for determining the character of the curd from cow's milk, etc. JOUR. DAIRY SCI. 6: 509. 1923.

(2) HILL, R. L. Soft-curd milk. Bulletin 227, Utah Agricultural Experiment Station. 1931.

(3) HILL, R. L. A decade and a half of soft-curd milk studies. Circular 101, Utah Agricultural Experiment Station. 1933.

(4) LADD, MAYNARD, M.D. Homogenized milk. Boston Med. and Surg. Jour. Vol. clxxiii, No. 1. Pp. 13–18. July, 1915.

(5) SHRADER, J. H., President National Dairy Products Corporation, Inc., 1403 Eutaw Place, Baltimore, Md. Personal correspondence.

(6) United States Patent Office—Patent Number 1,841,184. Process of Treating Milk. January 12, 1933.

(7) WALLACE, C. Soft-curd milk. Amer. Jour. Dis. Child. 44: 1143–1144. 1932. Cited by Hill (3).

(8) WASHBURN, R. M., AND JONES, C. H. Studies of the value of different grades of milk in infant feeding. Bul. 195, University of Vermont and State Agricultural College.

(9) WASHBURN, R. M. Soft-curd milk. The Milk Dealer 21(3). December, 1931.

(10) WEISBERG, S. M., JOHNSON, A. H., AND MCCOLLUM, E. V. Laboratory studies on the chemistry of soft curd milk. JOUR. DAIRY SCI. 16: 225–247. 1933.